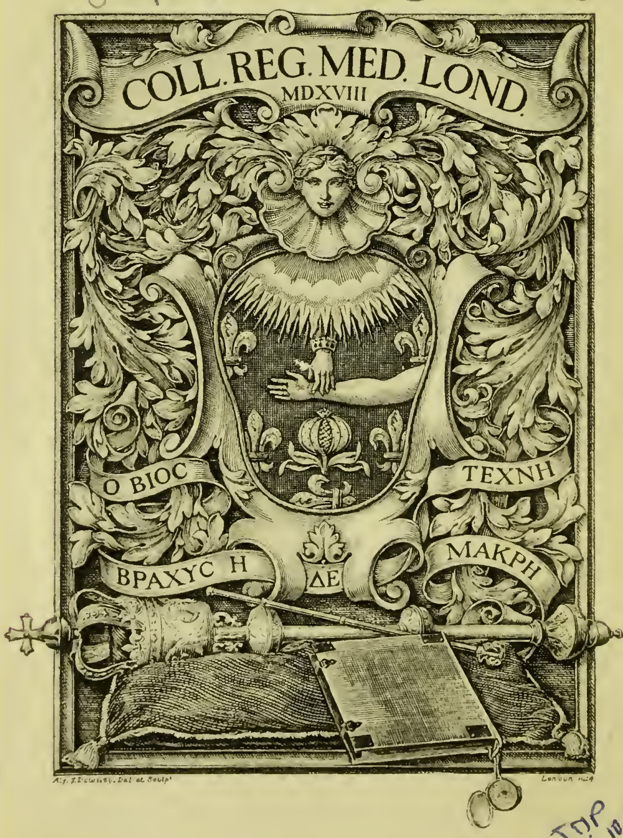
PHYSIOLOGICAL CHEMISTRY

OF

THE BRAIN

THUDICHUM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PHYSIOLOGICAL CHEMISTRY

OF

THE BRAIN.

A TREATISE

ON

THE CHEMICAL CONSTITUTION

OF

THE BRAIN.

BASED THROUGHOUT UPON ORIGINAL RESEARCHES.

BY

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1884.

TO THE

RIGHT HONOURABLE LORD SHERBROOKE,

OF SHERBROOKE,

IN GRATEFUL REMEMBRANCE OF THE HIGH-MINDED LIBERALITY WITH WHICH, DURING HIS TENURE OF THE OFFICE OF VICE-PRESIDENT OF THE COUNCIL, HE AIDED THE ORIGINAL INSTITUTION OF THESE RESEARCHES, AND OF THE ENLIGHTENED COUNTENANCE AND SUPPORT WHICH HE HAS CONTINUED TO GIVE THEM DURING THEIR PROGRESS,

This Treatise is Respectfully Inscribed

BY

THE AUTHOR.

PREFACE.

A CENTURY has nearly elapsed since the brain was for the first time made the object of physiological research, with the aid of the then newly discovered science of chemistry. But the attempt remained isolated at the time, and was renewed only at long intervals, mostly by inquirers who had no cohesion and little sympathy with each other, and particularly had no paramount object in the prosecution of such an inquiry. Now and then an eminent chemist just touched the problem, to leave it soon after he had recognised its inherent difficulties. During the last twenty years only were more frequent attempts made to approach the knowledge of the chemistry of the brain; but these results were so little satisfactory, that inquirers could not even think of proceeding to quantitative investigation of normal relations, much less of studying pathological phenomena. It was under such unpromising conditions that I undertook, about twelve years ago, to make some researches on this subject for the then Medical Department of the Privy Council. The progress which I was able to report in 1874 was sufficiently great to ensure acquiescence in the continuance of my efforts on the part of the former authority and subsequently of the Local Government Board, to whom the functions with regard to the administration of the Parliamentary grant for researches in aid of pathology and medicine, previously exercised by the Privy Council, were transferred. It soon became evident that the physiological inquiries, which had been intended as an introduction to pathological ones, would occupy far more time than had been anticipated. However, as the information grew, as the discoveries multiplied in number and gained in precision, no hesitation could be felt in following up the researches to a decided issue. Thus I came to trace the foundations of the

chemical constitution of the brain, the outlines of which I have the honour of laying before the medical profession and the general scientific public in the shape of the present treatise. The work is a systematic consolidation of all the researches on the subject which have been laid before Parliament in the Annual Reports of the Medical Officer of the Privy Council and Local Government Board respectively. The matter of the work is therefore public property, and under these circumstances I think it my duty to specially avow my responsibility for its contents.

I should not be surprised if some readers were, at the first glance, to think the subject recondite and its treatment heavy. Descriptions of complicated chemical processes are necessarily tedious to those who have no practical concern in their repetition or even knowledge. By some they will probably be treated with the consideration which Hellenic literature received before the Renaissance, and which has been recorded in the sentence: 'Græca sunt, non leguntur.' But I would beg all readers to take into patient consideration that I had to write a highly technical work on a most difficult subject, and to endeavour to make it acceptable, in such a form as it practically could have, to anatomists, physiologists, and pathologists, as well as to that principal part of the medical public which is engaged, like myself, in the practical pursuit of the profession.

However, to all readers who will enter upon the consideration of the subject with the intention of adding it to their previous stock of knowledge, I can promise the enjoyment of some intellectual pleasure. They will find the brain to be the most diversified chemical laboratory of the animal economy; they will find such numbers and varieties of hitherto unknown and most remarkable chemical principles taking part in such complicated chemical structures and processes, that the explanation of the mental phenomena, and of their aberrations under the influence of disease, seems much less difficult than it appeared before these discoveries.

I have not in this treatise entered upon any pathological considerations, although I have in several smaller publications shown the bearings which some of my discoveries have upon the practical study and treatment of diseases. Thus I have shown the morbid alteration of the nerve-marrow in locomotor ataxia, and the occurrence of a kind of glycohæmia to be intimately connected

with patholytic changes in substances of the group of cerebrosides. I could go further and unfold, *e.g.*, a chemical connection between the function of the liver and that of the brain, opening views into the pathology of the future and illuminating, though only with the disappointing brevity of an electric spark, regions as dark as those of general paralysis and melancholy. But I have (in early stages of my work) formed the resolution never to propound a generalisation on any subject before having proved the validity of all data obtainable by observation or experiment. And I must say that I have not yet found any subject in chemical biology on which, governed by that resolution, I should have liked to proceed to generalisation.

The principal reason for this abstention is the circumstance that the data or so-called facts available in chemical biology are as yet too incomplete, and therefore unsuitable for connected treatment except with the aid of hypotheses. This circumstance also affects the present treatise, and I have taken care to point out to the reader cases where my information was partial, or where no data at all were as yet available. In order, however, that the reader may not, from this avowal, come to an erroneous conclusion regarding my own estimate of the value of the researches communicated in this treatise, I undertake to assure him that they are of fundamental importance, and that all further developments in chemical neurology must start from them as a basis. I say this in view of the records of the work of all those who have grappled with the problem before me, and in kindness to all who may like to deal with it hereafter.

The literary discussions on subjects of brain chemistry which have taken place during the last few years, and in which I was obliged to take an active part, have had such an issue that I was happily enabled to exclude all controversy from the pages of my treatise. I have moreover endeavoured throughout to confine my matter to the narrowest space compatible with clearness of description and accuracy of demonstration, and it is owing to this resolution that I have omitted not only references to historical literature, but multifarious details of analytical figures, the reproduction of which would have enlarged the size and enhanced the price of the work without any corresponding advantage to the greater number of readers. Those, however, who value such matters will find a complete history of research in brain chemistry

detriment of the science of which, even by a short connection, their names have become permanent ornaments.

I am in hopes that several of the discoveries made on the brain, and communicated in this treatise, will be able to shed some light upon other subjects in biological chemistry, which are at present little understood. I have indicated some of my ideas on that subject in a few pages appended to the division on cerebral phosphatides, which give a short, comparative consideration of other phosphatides of the animal body.

Phosphatides are the centre, life, and chemical soul of all bioplasm whatsoever, that of plants as well as animals. Their chemical stability is greatly due to the fact that their fundamental radicle is a mineral acid of strong and manifold dynamicities. Their varied functions are the result of the collusion of radicles of strongly contrasting properties. Their physical properties are, viewed from a teleological point of standing, eminently adapted to their functions. Amongst these properties none are more deserving of further inquiry than those which may be described as their power of colloidation. Without this power no brain as an organ would be possible, as indeed the existence of all bioplasm is dependent on the colloid state.

I was preaching in the desert when, in 1866, I advanced the hypothesis, based upon numerous and deep investigations, that the fall of temperature in the collapse-stage of cholera, far below the normal, is due to the destruction of the colloid state in myoplasm, produced by the direct influence of the disease-cause. Now, in 1884, we can demonstrate to the eyes that there are bacteria or microzymes which have the power of liquifying colloids, while there are others which have not got that power. That the phosphatides may be capable of being affected by the former is very probable, particularly when it is observed how in cells bacteria congregate and multiply in close proximity to the nuclei. Now so-called softening of the brain consists in the first place merely in the loss of the colloid state on the part of a smaller or larger portion of nerve-marrow. The bacilli of tubercle may effect such a softening; but others may have the same power. The next stage after the fluidification is patholysis, the splitting up of the liberated immediate principles into their proximate nuclei, the same as those which we obtain by chemolysis. The two processes, the one in cholera, and the other in softening of

the brain, illustrate the acute and chronic form of loss of colloidation and of patholysis under the influence of microzymes.

Some phosphatides of the brain probably permeate the neuroplasm in a non-colloid dissolved liquid state, *e.g.*, amidomyelin; and this body has the remarkable property that while perfectly soluble in water, and non-colloid at the ordinary temperature and at temperatures approaching that of the animal body, it becomes colloid at temperatures between the normal and the highest fever heat. It is not impossible that some such change may be the cause of death in many febrile conditions, and in many cases of death from exposure to excessive heat, in which no adequate mechanical lesion can be discovered.

A few further examples may suffice to indicate some of the lines on which the practical consideration of diseases of the brain by the aid of its chemistry will, at least in the first instance, have to proceed. Many kinds of headache are probably due to intracranially brewed chemical poisons, or to poisons carried from the body to the brain by the blood, whether fermented in the body, or like alcohol, morphia, and fusel oil, formed out of the body. From such occasionally produced effects to the constant production of similar effects by a continued zymosis, be it now caused by organised or unorganised ferments, is not a great step. Many forms of insanity are unquestionably the external manifestations of the effects upon the brain-substance of poisons fermented within the body, just as the mental aberrations accompanying chronic alcoholic intoxication are the accumulated effects of a relatively simple poison fermented out of the body. These poisons we shall, I have no doubt, be able to isolate after we know the normal chemistry to its uttermost detail. And then will come in their turn the crowning discoveries to which all our efforts must ultimately be directed, namely, the discoveries of the antidotes to the poisons, and to the fermenting causes and processes which produce them.

THE AUTHOR.

11, Pembroke Gardens, W.,
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A TREATISE

ON THE

CHEMICAL CONSTITUTION OF THE BRAIN.

I.

INTRODUCTION.

The following work is a contribution towards a system of *chemical statics* of the brain, and treats in the first instance of brain matter as a whole, without separating white from grey matter; it isolates and scrutinises the several chemical constituents of nerve-matter, and endeavours, by the study and consideration of the peculiarities of each, and their combination, to obtain sufficient insight into normal and abnormal chemical functions to enable us in time to guide or to correct them.

It is thus found that this apparently so simple nerve-marrow, or neuroplasm, is a compound and mixture of a large number of heterogeneous principles arranged in such a manner as to vanish completely from appearance as chemical individuals; the compounds so interpenetrate each other that the resulting material is apparently homogeneous, during life completely so, when seen with high powers of the microscope, and although in death the homogeneity partly vanishes, yet even the appearance of the cylinder axis cannot be utilised chemically at present, and the isolation and recognition of any ingredients is entirely dependent upon most circumstantial chemical proceedings.

During these proceedings the first striking fact which meets the inquirer is that neuroplasm contains abundance of water. This, in conjunction with the peculiar manner in which the water

is contained, engenders a mobility of ultimate particles within certain limits of movement. It also gives penetrability by liquid diffusion, while excluding porosity and its capillary effects; by which means a ready nutrition by diffusion in one direction, and ready cleansing from the effete crystallisable products of life in another, are ensured. Consequently the brain as a whole is essentially made up of colloid matter, and may be compared to a colloid septum, on the one side of which is arterial blood and cerebrospinal fluid of the ventricles, on the other side, however, is cerebrospinal fluid of the arachnoideal space and venous blood. It follows from this that the large amount of water present in the brain is not there, so to say, mechanically only, like water in a sponge, and capable of being pressed out mechanically, but is chemically combined as colloid hydration water, or better, *water of colloidation.*

All soft organs of the body contain about three quarters of their weight of colloidation water, fixed within the organised limits of cells and fibres of all kinds. Cells and fibres are thus apparently not very different in respect of their mechanical condition from brain matter. Indeed, the difference is less in the manner of the condition than in the agents by means of which the condition is brought about. It is in effect mainly by the number and nature of these agents that brain matter is distinguished from other colloid tissues. It contains a considerable amount of an albuminous base. Whether this is distributed in the form of sheaths to fibres of nerve marrow, or whether it is laid into hollow spaces between the fibres, and acts as a cement—whether it is mixed or combined chemically with the rest of the matters constituting nerve marrow—must be discussed later. It may be present in all forms, but does not seem to be present in a liquid unattached form, as in serum. It appears that the bearing of soluble albumen when placed in presence of some of the peculiar brain matters changes from that which it ordinarily observes, no doubt by an influence of these brain matters amounting almost to combination. The bearing of albumen in the brain being thus seen to be governed by the matters peculiar to the brain, the present researches have in the first instance not been directed upon the condition and nature of the albumen in the brain, but upon the peculiar matters which seem not only to govern the albumen, but by their manifold chemical affinities assist promi-

nently in producing its singular mechanical arrangements, chemical function, and sensory and volitional action and reaction.

It is not asserted that these matters, or any of them, occur exclusively in brain matter, but being present in the nerves, they consequently can be extracted from all sensitive or contractile tissues. They, or some of them, also occur in aggregations of loose cells, such as the blood-corpuscles and pus-corpuscles ; others are present in serum, and others again in secretions, such as bile ; but the quantities in which these bodies are met with in parts and matters other than those of the nervous system is very far less than that in which they occur in the nervous system.

The great quantity of these matters occurring in the brain forms three groups ; the members of one contain at least five, sometimes six, elements, amongst which is phosphorus ; hence they may be termed PHOSPHORISED BODIES. The members of the second group contain four elements, amongst them nitrogen, but no phosphorus, and therefore are termed NITROGENISED BODIES. The members of the third group contain only three elements, carbon, hydrogen, and oxigen, present also in the other two groups, but neither phosphorus nor nitrogen, and may be termed OXIGENISED BODIES.

The group of the PHOSPHORISED BODIES contain the phosphorus in the form of phosphoric acid combined with from two to five organic compound radicles. As the earliest known body of this group, *lecithin*, yielded its phosphoric acid mainly in combination with glycerol, as glycerophosphoric acid, it was supposed that the phosphorised bodies, of which a number were theoretically admitted to exist, were constituted like the fats, by combination of compound organic radicles with the radicle of glycerol, in other words, that they were ethers of the alcohol glycerol, and contained the phosphoric acid as an inserted, and not as a fundamental radicle. But as we now know at least one phosphorised principle from the brain which does not contain any glycerol, and does therefore not yield, on chemolysis, any glycerophosphoric acid, but phosphoric acid merely without any attached organic radicle, we thereby obtain a new insight into the chemical constitution of the phosphorised substances altogether, and are under the necessity of subjecting their theory to a revision. According to the result of this revision the phosphorised substances are not glycerides at all, as commonly defined, and have nothing in

common with fats considered as glycerides, except that some of them contain certain fatty acids also present in fats, while they differ in physical and chemical properties widely from fats. In accordance with this new knowledge, I have termed the phosphorised substances *phosphatides*, that is to say, substances which are similar to (but not by any means identical with) phosphates, on the assumption that their basal or principal joining radicle is that of phosphoric acid, and that in this acid one, two, or three molecles of hydroxyl may be replaced by radicles of alcohols, acids, or bases, and that to a molecle formed by three such substitutions there may yet be attached, either by substitution of an element in a radicle itself already substituted (side-chain), or by addition with elimination of water from the added radicle, a fourth radicle, and that thus bodies of the following typical formulæ may be produced :

Phosphoric Acid.

(Phosphoryl) OP { HO (Hydroxyl). / HO (Hydroxyl). / HO (Hydroxyl).

Nonnitrogenised Phosphatide.

EXAMPLE : *Kephalophosphoric acid.*

(Phosphoryl) OP { Kephalyl. / Stearyl. / Glyceryl.

Nitrogenised Phosphatide.

EXAMPLE : *Lecithin.*

(Phosphoryl) OP { Oleyl. / Margaryl. / Glyceryl.
|
Neuryl.

Dinitrogenised Phosphatide.

EXAMPLE : *Amidomyelin.*

(Phosphoryl) OP { Acid radicle. / Alcohol radicle. / Alkaloidal or basic radicle (substituted).
|
Alkaloidal anhydride (attached as side-chain).

The bodies to which the foregoing formulæ may be applied contain the phosphorised radicle once, and may therefore be termed *monophosphatides;* but there are present in the brain and other protoplastic centres, bodies which contain the phosphorised radicle twice, and which may therefore be described as *diphosphatides;* the immediate principle representing this subgroup contains about seven per cent. of phosphorus, and may perhaps be constituted according to the following formula :

Dinitrogenised Diphosphatide.

EXAMPLE : *Assurin.*

$$\left.\begin{array}{l}\text{Acid radicle.}\\ \text{Alcohol radicle.}\\ \text{Alkaloidal radicle.}\end{array}\right\} \text{PO—OP} \left\{\begin{array}{l}\text{Acid radicle.}\\ \text{Alcohol radicle.}\\ \text{Alkaloidal radicle.}\end{array}\right.$$

I shall not in this place dilate upon the nonnitrogenised phosphatides, of which I have given a product as an example, although there are probably two such immediate principles contained in that part of the spirituous brain extract which is conveniently termed the buttery matter ; but I shall at once pass to a short consideration of the mononitrogenised monophosphatides, of which lecithin is the earliest known, and was, before the institution of my researches, the only one of which any closer knowledge existed.

In this subgroup nitrogen is to phosphorus in the proportion of one atom to one atom, a relation to be expressed by the formula $N : P = 1 : 1$. Of the educts of the brain four species with all their varieties belong to this subgroup, namely, the *lecithins*, *kephalins*, *paramyelins*, and *myelins*; a product also may be alluded to, obtained from a dinitrogenised educt by the loss of a nitrogenised radicle, namely, *sphingomyelic acid*. This latter contains no glycerol; the lecithins, and kephalins, and paramyelins probably contain glycerol, and yield glycerophosphoric acid; of the myelins, the constitution in this particular respect has yet to be ascertained.

Lecithin, originally obtained from and named after its occurrence in eggs, is only with difficulty evolved from the brain, on account not only of the many stages of the processes necessary for its isolation, but also on account of its readiness to decompose under certain conditions. This tendency is greatest in the presence of hydrochloric acid and platinic chloride, with which lecithin readily combines. It ceases almost entirely when lecithin

is combined with cadmium chloride, and the compound is dried. In the free state and in concentrated solutions, it has an apparently spontaneous tendency to change and assume colour, due no doubt to the known tenderness of the oleyl radicle by which it is characterised ; but the tendency to apparently spontaneous lysis into proximate nuclei is not so great as is supposed by most authors, and can now be almost entirely obviated by the improved processes for its isolation, which I shall have to describe below. It is, however, the most easily decomposable member of this group, and this lability furnishes a valuable key to the explanation of many changes in the sick body, which may arise, or have been proved to arise, from its decomposition. I have not hitherto found reason to suppose that there is a dinitrogenised lecithin present in the brain ; but in view of the dinitrogenised educts which will be described, the possibility of this occurrence must not be lost sight of.

I have given the explicit formulæ of the lecithins considered as phosphatides under the chapter relating to them ; the contracted formulæ are :

Oleo-palmito-glycero-neuro-phosphatide $= C_{42}H_{82}NPO_8$.

Oleo-margaro-glycero-neuro-phosphatide $= C_{43}H_{84}NPO_8$.

Oleo-stearo-glycero-neuro-phosphatide $= C_{44}H_{86}NPO_8$.

Of the second lecithin, there have been analysed the following salts :

Cadmium chloride salt - -	$C_{43}H_{84}NPO_8 + CdCl_2$.
Hydrochlorate - - - -	$C_{43}H_{84}NPO_8 + HCl$.
Hydrochlorate platino chloride -	$2(C_{43}H_{84}NPO_8 + HCl) + PtCl_4$.

In the chemolyses the *oleic*, *margaric*, *palmitic*, and *stearic* acids were isolated, oleic acid characterising the phosphatide ; stearic acid was always present in very small quantities only ; it was probable that of stearic acid the two isomers discovered in these researches may sometimes be present in lecithins. Regarding the reputed identity of margaric and palmitic acid, I still entertain some doubts, which have been rather increased than diminished by the discovery of the isomers alluded to. Besides these acids the lecithins always yielded glycerophosphoric acid and neurin. A specimen obtained from cadmium chloride salt soluble in cold

benzol, was recently analysed and found to answer to the foregoing description.

As the lecithin species is characterised by the presence of the radicle of oleic acid, so the *kephalin* species is characterised by the presence of the radicle of a peculiar acid, to which I have given the name of *kephalic acid.* This acid is even more changeable than oleic, and imparts its quality to all the compounds in which it is present. The change in these cases seems to be either acquisitive, *e.g.* by accession of oxigen, or intramolecular, *e.g.* by transposition of atoms, but does not appear to lead to lysis into proximate nuclei so easily as in the case of lecithin. The second fatty acid radicle in the case of the principal kephalin is stearyl, other radicles occurring only in extremely small quantities. The members of this subgroup vary in the amount of oxigen which they exhibit on analysis, in a manner so as to be apparently sharply characterised thereby. But this variability of the constituent oxigen may be transitional, and, on the whole, this remarkable feature, which none of the other phosphatides exhibit, requires much further investigation before it is adducible to any very precise theory.

A kephalin mav therefore be defined as kephalo-stearo-glycero-neuro-phosphatide, and represented by the formula

$$OP \left\{ \begin{array}{l} C_{17}H_{28}O_3 \text{ (kephalyl)} \\ C_{18}H_{35}O_2 \text{ (stearyl)} \\ C_3H_7O_3 \text{ (glyceryl)} \\ C_5H_{11}N \text{ (neuryl)} \end{array} \right\} = C_{43}H_{81}NPO_9 + \text{aq.}$$

To be a kephalin a phosphatide must contain the radicle *kephalyl*, or a homologue, which governs most of the properties of the compound; its peculiar properties prevail over those of the second acid.

A kephalin with palmityl, $C_{16}H_{31}O_2$, in place of stearyl, would have the summary formula, $C_{41}H_{77}NPO_9$ + aq.; a kephalin with margaryl, $C_{17}H_{33}O_2$ would be $C_{42}H_{79}NPO_9$ + aq. If there were several homologous kephalic acids, such as some analyses seem to indicate, then for a kephalyl of formula $C_{17}H_{30}O_3$, combined with either stearyl, margaryl, or palmityl, the foregoing formulæ would have to be increased by CH_2 each, so that the most complicated kephalin might contain 44 atoms of carbon. None of these hypotheses explain either the deficiency of hydrogen or the excess of oxigen

(namely, as compared to the theory derived from chemolysis) in the various kephalins and their compounds which have been analysed, and of which the following is a synopsis :

Kephalin (empirical formula) -	$C_{42}H_{79}NPO_{13}$.
Kephalin, perhaps - - -	$C_{42}H_{69}NPO_8 + 5H_2O$.
Kephalin cadmium chloride -	$C_{42}H_{79}NPO_{13} + CdCl_2$.
	(dissociates partially in watery reagents.)
Kephalin hydrochlorate platinum chloride - -	$2(C_{42}H_{79}NPO_{13} + HCl) + Pt\ Cl_4$.
Kephalin with amido-kephalin (mixture) - - -	$C_{42}H_{80}N_2PO_{13}$. $2(C_{42}H_{79}NPO_{13}$.
Oxykephalin cadmium chloride	$C_{42}H_{79}NPO_{14} + CdCl_2$.
Peroxykephalin - - -	$C_{42}H_{79}NPO_{15}$.
Peroxykephalin, diplumbic -	$C_{42}H_{75}Pb_2NPO_{15}$.
Kephaloidin - - - -	$C_{42}H_{79}NPO_{13}$.
Oxykephaloidin cadmium chloride - - - -	$2(C_{42}H_{75}NPO_{14}) + CdCl_2$.

There is little doubt of the existence of a dinitrogenised kephalin, termed *amidokephalin*, which was mixed to the extent of one-third nearly with a preparation of kephalin met with in the course of the researches. This body is less soluble in ether than kephalin. It is probably constituted analogously to the *amidomyelin* described in a special chapter. But as this amido-kephalin has not been finally examined, I am unable to give any further data concerning it.

Of the decomposition products of kephalin by chemolysis, I have already mentioned *kephalophosphoric acid*, under the non-nitrogenised monophosphatides. *Kephalic acid* is the principal chemolytic product of the process, in which the phosphatide is almost entirely severed. All its salts are soluble in ether and insoluble or little soluble in alcohol. It assumes, in the free state or in combination, a brown colour, from which it has not yet been possible to free it by any known process. The formulæ which can be constructed for the acid vary between $C_{19}H_{32}O_3$, and $C_{17}H_{28}O_3$, or $C_{17}H_{30}O_3$, and $C_{17}H_{32}O_3$. They are mainly derived from *barium salts*, which contain from 18·28 per cent. to 20·05 per cent. Ba. in some cases 19·29 per cent. to 19·89 per cent. Ba. The kephalates of barium, of calcium, and of lead, are brown precipitates.

Another chemolytic product of kephalin is *stearic acid*, of

melting-point 69·5; this was examined in the free state, and as barium and lead salt.

Stearic acid - - -	$C_{18}H_{36}O_2$.
Barium stearate - -	$2(C_{18}H_{35}O_2)Ba$.
Lead stearate - -	$2(C_{18}H_{35}O_2)Pb$.

Glycerophosphoric acid was isolated from kephalin as lead salt, calcium salt, $C_3H_7CaPO_6$, and in the new form of *acid calcium salt*, $C_6H_{16}CaP_2O_{12}$; as barium salt, $C_3H_7BaPO_6$; this could be obtained crystallised, monohydrated, and as alcoholohydrate, in which latter state one molecle of *acid* glycerophosphate of barium, composed similarly to the acid calcium salt, contained at least three molecles of alcohol and six of water.

Kephalin further yielded *neurin* and another base, perhaps derived from the former. The second base in amidokephalin was not ascertained.

The third subdivision of this subgroup is represented by *paramyelin*, a phosphatide which contains probably glyceryl, neuryl, and an oleo-cholide radicle hitherto unknown. It strikes with oil of vitriol and sugar syrup an immediate deep purple colour; the mixture of acids obtained by chemolysis with barita does the same in a more intense manner. Paramyelin is a white, firm, solid body, crystallising from boiling spirit in plates and needles. It combines with cadmium chloride, and the solubility of this compound in hot benzol, and its insolubility in cold benzol, afford facilities for the isolation of the body. A specimen of cadmium chloride salt from human brain gave on preliminary analysis $C_{34}H_{68}NPO_8 + CdCl_2$; another from oxbrain, $C_{38}H_{76}NPO_9 + CdCl_2$. It is not maintained that these compounds were unitary and did not contain an admixture of a small quantity of one or more principles similar to paramyelin, *i.e.*, paramyelins differing from each other in the item of the second acid radicle. Paramyelin also forms a hydrochlorate platinochloride salt.

While lecithin and paramyelin exhibit mainly alkaloidal functions, and kephalin shows alkaloidal and acid functions on a wide area, the fourth subdivision or species of the mononitrogenised monophosphatides, the *myelins*, exhibit mainly acid properties. The representative *myelin* is a firm compound, and combines with lead like a dibasic acid; that is to say, admits a didynamic atom of lead in place of two atoms of hydrogen. On the other hand, it

does not combine with cadmium chloride as do lecithin, kephalin, paramyelin, amidomyelin, and sphingomyelin. This peculiarity seems to indicate a peculiar constitution, of which, without exhaustive chemolyses, no account can be given, but which may be such as to necessitate the separation of myelin from the other three members of the subgroup, and its allocation to a subgroup, of which it would be the peculiar representative. Myelin and its compounds on analysis have yielded the following formulæ :

Myelin in lead salt - - -	$C_{40}H_{75}NPO_{10}$.
Myelin free - - - -	$C_{39}H_{77}NPO_{9}$.
Myelin lead - - - -	$C_{40}H_{73}PbNPO_{10}$.

There may, perhaps, be myelins varying in carbon from 39 to 44 atoms.

I now come to the subgroup of *dinitrogenised monophosphatides*, of which I had given a preliminary notice in my researches of 1874 under the name of *amidomyelin* and *apomyelin*, but of which the former has been isolated only lately, while the latter has been more fully investigated, and classified with a body isolated and investigated under the name of *sphingomyelin*. Amidomyelin completely bore out the theory of the composition which I had assigned to certain compounds of mixtures of brain-educts with platinum and cadmium chloride ; out of such mixtures amidomyelin on the one, and lecithin and paramyelin on the other hand, were isolated first as compounds, afterwards in the free state. If any argument were needed to justify the analyses of the mixtures alluded to, analyses to which no fairminded inquirer would attribute any other character than that of reconnoitring proceedings, the success which has been induced by their results would be sufficient. Apomyelin was found to be a genuine educt and immediate principle of the brain.

Of amidomyelin I have, therefore, practically proved the existence and individuality by direct isolation and analysis. But I have not had time to ascertain anything about its constitution by chemolysis of the pure substance. There are, however, some data available for a preliminary view of at least some of the radicles likely to be met with in the body, in a research on the chemolytic products of a cadmium precipitate, which in the relative research I have described as the principal cadmium salt.

Amidomyelin is analogous to sphingomyelin and apomyelin in this, that it contains two atoms of nitrogen upon one atom of

phosphorus; these two atoms of nitrogen are disposed in two different radicles, and influence the character of the compound so that it presents itself as a diacidic base, or a dipolar alkaloid. In the latter quality it combines with cadmium chloride in two ratios, the fully saturated compound, which is that most commonly obtained, containing a little more than 30 per cent. of cadmium chloride.

Amidomyelin has been proved to exist in five forms, one of isolation, four of combination.

Amidomyelin - - - - - -	$C_{44}H_{88}N_2PO_9$.
Amidomyelin hydrochlorate - -	$C_{44}H_{88}N_2PO_9 + HCl$.
Amidomyelin monocadmium chloride -	$C_{44}H_{88}N_2PO_9 + CdCl_2$.
Amidomyelin dicadmium chloride -	$C_{44}H_{88}N_2PO_9 + 2(CdCl_2)$.
Amidomyelin hydrochlorate platinum chloride - - - - -	$2(C_{44}H_{88}N_2PO_9 + HCl) + PtCl_4$.

The second subgroup of the diamidated monophosphatides includes *sphingomyelin* and *apomyelin.* Sphingomyelin has been much studied and chemolysed, and has given not only the fundamental information upon which the hypothesis of the phosphatides is based, but also the first knowledge of a number of compound radicles new to science. It is not maintained that the knowledge of the bodies, acids, alkaloids and alcohols, in the shape of which they appear after chemolysis of the principle, is definitely rounded off. For in its prosecution, conditions of the utmost difficulty and complexity were met with, arising mainly from homology and isomerism. Thus one product was a fatty acid of the composition expressed by the formula $C_{18}H_{36}O_2$, being the *third isomer* of stearic acid discovered in my researches, *sphingostearic acid,* fusing at 57°, therefore at almost the same interval below, as the second isomer of stearic acid, namely, *neurostearic acid,* fusing-point 84°, fuses above the fusing-point of ordinary stearic acid, which latter melts at 69·5°. It is evident from this that henceforth a fatty acid from the brain can be diagnosed neither by its elementary composition alone, nor by its melting-point alone, but that a knowledge of both is required to approximately fix its nature; an accurate diagnosis requires the knowledge of all physical and chemical properties and of reactions. More particularly a mere melting-point determination of a sample of fatty acid from the brain has, by itself, no diagnostic value whatever, and particularly can no longer be used in the attempt to make out, by the well-known tables of Heintz, the quantities of different ingredients in

mixtures of which certain melting-points are supposed to be accurate exponents.

How wonderfully the phenomenon of isomerism complicates brain research and biological research in general will become still more apparent by the following. Another product of the chemolysis of sphingomyelin is an alcohol, *sphingol*, of the empirical formula $C_9H_{18}O$, or $C_{18}H_{36}O_2$, which, on the supposition that the latter formula expressed its atomic weight, would be the *fourth isomer* of stearic acid.

A third product of the chemolysis of sphingomyelin is an alkaloid closely resembling the sphingosin of the cerebrosides, $C_{17}H_{35}NO_2$, but showing a little more carbon and hydrogen, so as to answer to the formula $C_{20}H_{41}NO_2$. To these three radicles, a molecle of neuryl, $C_5H_{11}O$, is yet attached, so that on the basis of its chemolysis we are able to attribute to sphingomyelin three somewhat different formulæ of constitution, in each of which three terms are certain, while of the remaining two terms one is certain as regards its percentic composition, but uncertain as regards its atomic weight; while the other, namely the second nitrogenised radicle, is not yet sufficiently well defined to afford much aid to synthetical calculations. The smallest formula gives a total of $C_{52}H_{104}N_2PO_7$, while the largest leads to a formula with 61 Carbon; but all empirical formulæ for sphingomyelin, which we shall have to consider below, lead to the necessity of assuming the presence in the free body of a few atoms of water, which are not accounted for by the sum of the products of the chemical cleavage.

It is certain that the smallest formula just given, and which is almost identical with the formula for apomyelin formerly given, is only a type, and that there are varieties in which either the alkaloid or the fatty acid, or the alcohol differ from those just formulated probably by $- +CH_2$, or $- +nCH_2$. The increase in CH_2 of the alkaloid obtained by chemolysis beyond the quantities demanded by the formula of sphingosin may be due to the admixture of a small quantity of a body being a compound of sphingol, $C_{18}H_{36}O_2$, and sphingosin, $C_{17}H_{35}NO_2$; total, $C_{35}H_{69}NO_3+H_2O$. If all these data are constant, then we cannot doubt that the limits of error are confined between very small dimensions.

The typical sphingomyelin crystallises well in microscopical plates, combines with cadmium chloride in two ratios, the compounds being crystalline, and gives up one nitrogenised radicle

on limited chemolysis, namely the loosely bound neurin, $C_5H_{13}NO$, leaving an acid which contains all the phosphorus of the sphingomyelin together with half the original nitrogen, and in which, therefore, $N : P = 1 : 1$. The formula of the acid, a produced mononitrogenised monophosphatide, from the C_{53} sphingomyelin is $C_{48}N_{95}NPO_{12}$, and its name may be *sphingomyelic* acid.

The following is a synopsis of the bodies belonging to this subgroup and their compounds and chemolytic products :

Educts.

Sphingomyelin (ox) - - - -	$C_{52}H_{104}N_2PO_9 + H_2O$.
Apomyelin (man) - - - -	$C_{54}H_{109}N_2PO_9$.
Sphingomyelin (theory from chemolysis)	$C_{58}H_{115}N_2PO_7 + 2H_2O$.
Sphingomyelin cadmium chloride -	$C_{51}H_{99}N_2PO_{10} + CdCl_2$.
Do. dicadmium chloride -	$C_{51}H_{99}N_2PO_{10} + 2(CdCl_2)$.

Derivates by Chemolysis.

Sphingomyelic acid - - - -	$C_{48}H_{95}NPO_{12}$.
Sphingosin - - - - -	$C_{17}H_{35}NO_2$.
Base (as sulphate) - - - -	$2(C_{20}H_{41}NO_2) + H_2SO_4$.
Sphingol - - - - - -	$C_{18}H_{36}O_2$ or $C_9H_{18}O$.
Sphingostearic acid (m.p. 57°) - -	$C_{18}H_{36}O_2$.
Neurin - - - - - -	$C_5H_{13}NO$.
Nitrogenised product - - -	$C_{35}H_{69}NO_3$.
Phosphoric acid - - - -	H_3PO_4.

The diamidated phosphatides yield hydrochlorates which crystallise from anhydrous solvents in the presence of excess of acid in exquisite form and purity; but they are not stable in the presence of watery reagents, and yield hydrochloric acid to solvents at every recrystallisation, so as to become almost free from the acid by mere repetition of this process. When the hydrochlorates are produced from the cadmium salts, they are of course mixed with double the quantity of hydrochloric acid necessary for neutral salts; and at this point great care is required so to manage the necessary warming of the mixture, that the salt may be formed without any very great part of it being chemolysed under the influence of the redundant hydrochloric acid.

The diphosphatide to which I have already alluded, in which $N : P = 2 : 2$, and to which I have given the name of *assurin* (from the Assyrian God), has in its platinum chloride compound the formula $C_{46}H_{94}N_2P_2O_9$. It is at present the most phosphorised body of this class known, while one of the least phosphorised,

relatively to the nitrogen, is a phosphatide educed from the bile as a crystallised platinum chloride salt in which N : P = 4 : 1, and which has a formula by which it is characterised as a tetrapolar alkaloid, the four basic poles corresponding to the four nitrogenised radicles with which the body is endowed.

Thus I have shown that the phosphorised educts of the brain are a class of bodies with numerous genera, each genus having again species and varieties; that they are of greatly varying chemical construction and function, and that they are so distributed as to show that they are specifically concerned in the most intimate parts and processes of protoplasmic life.

The presence of water diminishes the number or avidity of affinities in all phosphatides; it combines itself with these bodies in a peculiar manner, by which they show their character as colloids, and it afterwards dissolves them, some in a perfect, others in a peculiar and imperfect manner. When this hydric colloidation is at a maximum, the tendencies to decomposition seem to be at a minimum. The watery solutions do not decompose in stoppered bottles for many months; only after six months some specimens so kept showed signs of decomposition or putrescence. It seems therefore that water satisfies some of those affinities, and what is most remarkable, its influence increases and diminishes with the mass which is present and capable of acting, so that it displaces, when in quantity, other combinants, but when these other combinants prevail, water is itself displaced, and the colloid state instantly disappears. In the dilute watery solutions of the phosphorised bodies therefore almost every reagent soluble in water, when added in a certain excess, produces a precipitate, which contains the reagent in combination. But when water, or any watery solvent capable of dissolving the combined reagent, is brought in contact with the compound, the compound immediately dissociates; the reagent passes into the water, *pari passu* as the phosphorised body passes into the hydrated colloid state, and if the influence of the water is continued by renewal, the process terminates by a complete separation; the phosphorised body is again free and pure, and swells and dissolves as at first.

The reagents with which the phosphorised bodies are thus able to combine, and from which they are dissociated by water, are acids, alkalies, and salts. The phosphorised bodies therefore possess alkaline affinities (for acids), acid affinities (for alkalies),

alkaloid affinities (for salts); all those affinities are overcome by water in quantity, but the affinities for water are overcome by some metallic oxides, such as of lead, copper, manganese, iron, and even to a slight extent by lime and potash; these latter compounds are dissociated only by strong mineral acids, and the compounds can then be dialysed out. All other combinants separated by water alone can be completely removed from the phosphorised substances by dialysis on vegetable parchment.

We have therefore here a diversity of affinities such as is not possessed by any other class of chemical compounds in nature at present known; and the exercise of these affinities being greatly influenced by the mass of reagent, and the mass of water which may be present, the interchange of affinities may produce a perfectly incalculable number of states of the phosphorised and consequently of brain-matter. This power of answering to any qualitative and quantitative chemical influence by reciprocal quality or quantity we may term the state of *labile equilibrium;* it foreshadows on the chemical side the remarkable properties which neuroplasm exhibits in regard of its vital functions.

From this it also follows that neuroplasm (if only as characterised by the phosphorised bodies) must yield obedience to every, even the slightest external chemical influence, which may reach it by way of the blood. It must take up metals, acids, salts, alkalies, and alkaloids presented by the blood; it can retain only oxides when the serum is again free from the combinants; a watery serum will wash the brain, a more watery one will make it swell and displace mechanically within physiological limits what it can; a still more watery one will make the brain dropsical and produce some of the conditions of mechanical pressure on the brain. All these processes are the necessary consequences of the affinities of the phosphorised substances, and these being known, the phenomena could be predicted, if they were not sufficiently known as phenomena, though hitherto destitute of an explanation. Thus the so-called brain-fungus, the continued protrusion of brain-matter through apertures of the skull produced by mechanical injuries, may in certain cases find a physical explanation in simple excessive hydration of the phosphorised (and nitrogenised) principles, producing general intra-cranial pressure.

These few examples show that the acquisition of chemical statics leads almost necessarily and very easily to chemical dyna-

mics of the brain; and these will in their turn furnish data for physiological and pathological conclusions. But these deducive arguments must be most sparingly and cautiously used, until the statics are in a state of perfection and completeness. To argue too far from incomplete data would, seeing the history of biological chemistry during the last thirty years, be a deplorable error.

The NITROGENISED NONPHOSPHORISED substances of the brain imitate in many respects, but with little intensity, the properties of the phosphorised. Thus some have the affinity for water to the extent of swelling to gelatinous masses, but they do not go to the state of apparent solution, and do not pass paper filters under any pressure. Some are insoluble in ether, cold benzol, cold alcohol, some soluble; all dissolve in hot benzol or alcohol, and are deposited on cooling almost entirely. Therefore as compared to the phosphorised bodies, their chemical character is slight solubility; from their hydrated colloid state they are also reduced to a more compact and combined one by many acids, alkalies, and salts, and they retain many oxides in combination. Water has the same dissociating influence upon these as upon the compounds of the phosphorised principles. The nitrogenised bodies are all firm compounds, and do not easily oxidise or decompose. Their atoms show very little tension; but they possess substitution poles, where hydrogen is replaceable by metalloids, or compound radicles; their compounds with salts, oxides, or acids, are so unstable as not to admit at present of quantitative definition.

This group includes six great subgroups, of which the first four have a great number of features in common, while the two last subgroups are dissimilar to the former, and much more simple as regards chemical constitution.

The first subgroup is that of the *cerebrosides*, or bodies which contain a peculiar sugar, *cerebrose*, in which different radicles of acids and alkaloids (it is not known whether of alcohols also, in some cases) are inserted. Thus, of *phrenosin*, $C_{41}H_{79}NO_8$, the definition and formula of constitution are the following:

Neurostearo-Sphingoso-Cerebroside.

$$\begin{array}{r} \text{Saccharoid radicle} \\ C_6H_{10}O_4 \text{ (cerebrosyl)} \end{array} \left\{ \begin{array}{l} \qquad \text{Fatty acid radicle} \\ C_{18}H_{35}O_2 \text{ (Neurostearyl).} \\ \qquad \text{Alkaloid radicle} \\ C_{17}H_{34}NO_2 \text{ (Sphingosyl).} \end{array} \right.$$

The derivates by chemolysis and synthesis of products are the following:—*Cerebrose*, a crystallised sugar, isomer of glucose, and like this dextrorotatory, reducing copper solution, and tasting sweet, of formula $C_6H_{12}O_6$; *Neurostearic acid*, an isomer of stearic acid, fusing at 84°, of formula $C_{18}H_{36}O_2$; *Neurostearic ether*, $C_{20}H_{40}O_2$, or $(C_2H_5)C_{18}H_{35}O_2$, produced during chemolysis of phrenosin in alcohol by sulphuric acid, can be distilled unchanged in vacuo; *Sphingosin*, an alkaloid, $C_{17}H_{35}NO_2$; as *sulphate*, $2(C_{17}H_{35}NO_2)H_2SO_4$, insoluble in absolute alcohol; or *hydrochlorate*, $C_{17}H_{35}NO_2HCl$, soluble in water; *Psychosin*, $C_{23}H_{45}NO_7$, being the cerebroside of sphingosin, a body having alkaloidal properties, but less pronounced than those of sphingosin; *Æsthesin*, a compound of sphingosin and neurostearic acid less water, $C_{35}H_{69}NO_3$. Under the influence of heat phrenosin yields a *caramel*, $C_{41}H_{71}NO_4$, by the loss of four molecles of water; by a similar reaction *psychosin* also yields a caramel of the formula $C_{23}H_{37}NO_3$; these caramels are brown, insoluble in spirit, soluble in ether.

Synopsis of cerebrosides and derivates:

Phrenosin (educt) - - - -	$C_{41}H_{79}NO_8$.
Derivate by dehydration; caramel -	$C_{41}H_{71}NO_4$.
Derivate by substitution: Nitrited phrenosin nitrate - - -	$C_{41}H_{79}N_3O_{13}$, or $C_{41}H_{78}(NO_2)NO_8+HNO_3$.

Derivates by cleavage (chemolysis) and synthesis of products:

Cerebrose - - - - - -	$C_6H_{12}O_6$.
Neurostearic acid - - - -	$C_{18}H_{36}O_2$.
Ditto ether - - - - -	$C_{20}H_{40}O_2$ or $(C_2H_5)C_{18}H_{35}O_2$.
Sphingosin - - - - -	$C_{17}H_{35}NO_2$.
Ditto sulphate - - - -	$2(C_{17}H_{35}NO_2)+H_2SO_4$.
Ditto hydrochlorate - - -	$C_{17}H_{35}NO_2+HCl$.
Psychosin - - - - - -	$C_{23}H_{45}NO_7$.
Caramel of psychosin - - -	$C_{23}H_{37}NO_3$.

The second cerebroside, and accompanying phrenosin, is *kerasin*, a body crystallising in microscopic filamentous masses, which are very voluminous, and enclose mechanically great volumes of spirit. Although the knowledge of this body has been greatly advanced lately by the discovery of processes for its separation from sphingomyelin, and more particularly from a body which I shall describe later under the name of *krinosin*, yet circumstances did not allow me to finally fix by chemolysis its rational constitution in the same manner as this has been done

for phrenosin. Kerasin may, like phrenosin, comprise a number of analogously constituted bodies, of which the most probable formulæ are :

Educts :

Kerasin - - - - -	$C_{42}H_{85}NO_8$.
Ditto - - - - -	$C_{44}H_{89}NO_8$.
Ditto - - - - -	$C_{46}H_{92}NO_9$.

Products by chemolysis :

Psychosin (as sulphate) - -	$2(C_{23}H_{45}NO_7)+H_2SO_4$.
Cerebrose - - - - -	$C_6H_{12}O_6$.
Fatty acids of the formula - -	$C_nH_{2n}O_2$.

Both kerasin and phrenosin are neutral bodies, and do not combine with acids, alkalies, and salts in stoichiometric proportion.

The *cerebrinacides*, or cerebrin bodies which combine with lead and other bases, are as yet little known. They will yet require long and important researches, not only because they are numerous, but more particularly because they occur mixed with a class of *sulphurised bodies*, of which I give a preliminary sketch below. It is probable that the first cerebrinacide to be considered—namely, *cerebrinic acid*—may be a cerebroside, in which three hydroxyls are replaced (in phrenosin and kerasin only two hydroxyls are thus replaced), two by fatty acid radicles, one by an alkaloid radicle. The fact that cerebrinic acid became dehydrated like phrenosin under the influence of heat, assumed a brown colour, lost its solubility in spirit, and acquired a new solubility in ether—in short, that it became changed in the same manner as a saccharide is changed when it passes into a caramel—supports this surmise. Whether others of the cerebrinacides are cerebrosides can at present be neither asserted nor denied. Several of the cerebrinacides which have been isolated, such as spherocerebrin and the (according to quantity) principal cerebrinacide, are distinguished from the cerebrosides and cerebrinic acid by their containing a much larger proportion of oxygen than these bodies. The following is a synopsis of these bodies as far as they are isolated, together with their preliminary empirical formulæ :

Cerebrinic acid - - - -	$C_{59}H_{113}NO_9$.
Caramel of cerebrinic acid - -	$C_{59}H_{105}NO_5$.
Spherocerebrin - - - -	$C_{58}H_{123}NO_{17}$.
Cerebrinacide, principal - -	$C_{55}H_{113}NO_{21}$.

There are a number of subordinate cerebrinacides waiting for closer investigation.

Of the *cerebrosulphatides* I have hardly done more than demonstrated the existence. No representative body has been isolated to the extent of being free from phosphorus and cerebrinacide; indeed, it must remain a question whether there are not bodies which contain sulphur and phosphorus at the same time. The most concentrated preparation of cerebrosulphatide which I have succeeded in producing contained 4 per cent. of sulphur.

We now progress to the consideration of an entirely new series of bodies, which are so constituted, and exhibit such properties, that they may perhaps be described as *nitrogenised fats* or *amidolipotides.* Although they occur mixed with the cerebrosides, nevertheless they are at once demonstrated not to be such by the low amount of oxygen which they contain. Of these, the first is *bregenin* (from the Low German 'bregen,' head or brain), which is easily soluble in cold ether, crystallizes, and has the formula $C_{40}H_{81}NO_5$. The second one is *krinosin* (from the Greek word for hair, the wavy crystals resembling a mass of tangled long hair), $C_{38}H_{75}NO_5$, insoluble in cold, easily soluble in boiling ether. Homology of these two bodies, which is suggested by a comparison of the formulæ—

Bregenin - - - - -	$C_{40}H_{81}NO_5$
Krinosin - - - - -	$C_{38}H_{77}NO_5$

may not be assumed, as the one with the higher number of carbon atoms fuses at a much lower temperature than the one with the lesser number of carbon atoms.

Of the group of alkaloids occurring in the brain, *hypoxanthin* and its compounds were known to science, but isolated with greater precision from brain, particularly by the aid of phosphomolybdic acid. The following bodies were produced and analysed:

Hypoxanthin - - - -	$C_5H_4N_4O$.
Ditto hydrochlorate - - -	$C_5H_4N_4O + HCl + H_2O$.
Ditto with platinum chloride -	$2(C_5H_4N_4O + HCl) + PtCl_4$.
Ditto nitrate - - - -	$C_5H_4N_4O + HNO_3$.
Ditto with silver nitrate - -	$C_5H_4N_4O + AgNO_3$.
Ditto with silver - - -	$C_5H_4N_4O + Ag_2$.

The other alkaloids contained in the brain are either much more complicated or more simple than hypoxanthin. I have distinguished these, pending further inquiry, the *second alkaloid* as *gladiolin*, and the *third alkaloid* as *tennysin*; and regarding them little more can be stated than the methods by which they were

isolated, and the elements of which they seem, empirically, to be composed :

Gladiolin (second alkaloid)	$C_{15}H_{20}N_6O_5$.
Ditto hydrochlorate gold chloride - - -	$C_{15}H_{20}N_6O_5 + HCl + AuCl_3$.
Tennysin (third alkaloid)	C_3H_5NO.

The *amidoacids* and *amides*, represented by *leucin* and *allied principles*, and *tyrosin*, bodies otherwise well known in science, were isolated from healthy brain for the first time in the course of these researches :

Leucin - - - -	$C_6H_{13}NO_2$.
Tyrosin - - - -	$C_9H_{11}NO_3$.

Urea was isolated from brain and cerebrospinal fluid, particularly in disease. In cases of cholera I found that the cerebrospinal fluid contained as much urea as healthy human urine—namely, about 2 per cent.

The group of *oxigenised nonnitrogenised principles* consists mainly of *alcohols* and *organic acids.* The *alcohols* have very slight combining powers. The most prominent of these, as regards both quantity and appearance, is *cholesterin.* Discovered originally in human gallstones, and erroneously believed to be a fat, this principle received the inappropriate name which hides its significance and character. Insoluble by itself in water, it is probably dissolved in neuroplasm by means of the phosphorised substances. Its bearing is therefore governed, and its *rôle* determined to a great extent, by these matters. But its intrinsic chemical dynamis is probably also independent to some extent, as its atomic weight is very high. As an alcohol it is monodynamic, and if it did combine with acid radicles, naturally would therefore give rise to one class of ethers only. If such compounds exist in the brain, they must be in a state of high atomic tension, and fall to pieces by the mere fact of the application of solvents.

Some data seem to point to the existence of a second alcohol by the side of cholesterin, and isomeric or homologous to it, but the hypothesis lacks definite proof. I have sometimes found cholesterin fusing at 137°, the point at which some earlier writers supposed cholesterin always to fuse. But since the fusing-point of the principal cholesterin is certainly 145°, the lower fusing-point of 137° may belong to an isomer or homologue, and this I propose to term *phrenosterin.*

The bodies which may conveniently be considered as forming the *subgroup of carbohydrates* are *inosite*, the isomer of dextroglucose, discovered in and bearing its name from muscle, and *glycogen*, discovered in the liver and other organs. It has seemed to me that the inosite from human brain was somewhat different from that of the ox; at least, while the latter easily gave a compound with cupric oxide of the formula $C_6H_{12}O_6 + 3CuO + 3H_2O$, the former gave no such precise combination, and reacted in a peculiar manner. Some have supposed inosite to be the material from which, by a post-mortem metabolism, the lactic acid of the brain took its origin. But since it has been shown by others that inosite by ferments yields ordinary zymolactic acid, whereas, as I have shown, the lactic acid of the brain is always and exclusively the optically active paralactic or sarkolactic acid originally discovered in flesh, this apprehension has become obsolete.

The names and formulæ of the bodies belonging to this subgroup are the following:

Cholesterin, m.p. 145° - -	$C_{26}H_{44}O$.
Phrenosterin, m.p. 137° - -	$C_{26}H_{44}O$. (?)
Inosite - - - - - -	$C_6H_{12}O_6$.
Ditto tricupric trihydrate -	$C_6H_{12}O_6 + 3CuO + 3H_2O$.

The subgroup of the *nonnitrogenised organic acids* is represented by at least four different principles—*formic*, *sarkolactic*, *succinic*, and a tribasic acid which may perhaps be termed *oxyglyceric*. *Formic acid* is present in such small quantity only that it can just be recognised in the distillate by reactions. But *sarkolactic acid* occurs in quantities up to 1 per mille of the fresh brain substance. It was thus possible to subject this body to a more intimate study, and describe its properties with great precision. *Succinic acid* was separated and identified in a satisfactory manner. The fourth acid, *oxyglyceric*, was isolated and studied mainly by the aid of its silver salt. The following is a synopsis of these acids, their formulæ, and some of their compounds:

Formic acid - - - - -	CH_2O_2.
Sarkolactic acid - - -	$C_3H_6O_3$.
Ditto zinc salt, hydrate - -	$Zn(C_3H_5O_3)_2 + (H_2O)_2$.
Ditto calcium salt, anhydrous -	$Ca(C_3H_5O_3)_2$.
Succinic acid - - - -	$C_4H_6O_4$.
Oxyglyceric acid - - -	$C_3H_8O_4$.
Ditto silver salt, tribasic - -	$C_3H_5Ag_3O_4$.

The *albuminous substances* of the brain may be considered as *nitrogenised sulphatides*, inasmuch as sulphur is an essential constituent; if bodies like casein were present in brain, as has been supposed by some inquirers, they might, like the casein of milk, be true *nitrogenised sulphatide-phosphatides;* if connective tissue were present, it would perhaps come under neither definition. These substances are as yet very little known, for they could be examined with advantage only after all the substances described in the foregoing pages were known and could be separated. But in this process the albuminous bodies undergo such important alterations that the difficulty of their study is thereby only altered in kind, but not diminished in amount. With due consideration to this circumstance, they were in the first instance studied by chemolysis, and have yielded very remarkable results. The derivates by chemolysis included the following:

Albuminol	$C_{16}H_{23}NO_3$. (?)
Volatile sulphurised body	——
Ammonia and compound ammonias	NH_3.
Carbonic acid	CO_2.
Fatty acid	——
Oxalic acid	$C_2H_4O_4$.
Phosphoric acid	H_3PO_4.
Sulphurous acid	SO_2.
Acetic acid	$C_2H_4O_2$.
Alkaloids: Leucein	$C_6H_{11}NO_2$.
Alkaloids: Second alkaloid	$C_{12}H_{25}N_3O_7$.
Alkaloids: Do. copper compound	$C_{12}H_{23}CuN_3O_7$.
Alkaloids: Third alkaloid	——
Leucin	$C_6H_{13}NO_2$.
Ditto copper compound, monocupric dileucin	$2(C_6H_{12}NO_2)Cu$.
Glycoleucin, isomer of leucin	$C_6H_{13}NO_2$.
The cupric compound is also isomeric with the cupric compound of leucin	$2(C_6H_{12}NO_2)Cu$.
Tyrosin	$C_9H_{11}NO_3$.
Tyrosin mercurous chloride mercuric oxyde	$2(C_9H_{10}NO_3 + HgCl) + HgO$.

With this list the number of products is by no means exhausted. Only few quantations have as yet been made, so that the materials for drawing any conclusion regarding the constitution of the albuminous substances of the brain are not yet to hand.

The *inorganic ingredients* or *mineral substances* of the brain are distributed amongst its juices and solid ingredients in a very remarkable manner, as will be discussed more fully below. These bodies were formerly studied by means of analyses made on materials obtained by combustion of the brain as a whole. This proceeding had two sources of fallacy connected with it, which greatly diminished the value of its results. In the first place, the phosphoric acid produced by the destruction of the phosphatides (in which, of course, phosphoric acid is in *organic* combination) was calculated as *mineral* phosphoric acid, it being found partly in combination with bases, partly free. Owing to its being in excess over the whole of the saturating power of the bases, it expelled all volatile acids, such as carbonic, and sulphuric, and chlorine, and was not even itself entirely preserved from the reducing influence of the glowing charcoal, which volatilized a portion of it as phosphorus. This inconvenience was only partially avoided by the use during combustion of caustic baryta or barium nitrate. In fact, by no method as yet proposed could *the sulphur or phosphorus in organic combination* be kept separate from that in inorganic combination. Some progress has, however, been made by the separation of the soluble educts from the insoluble ones, and from the interstitial juices; and as each of these complex substances retains mineral matters peculiar to itself, while the phosphatides can be almost completely excluded by precipitation with acid from their solution or suspension in water, it is feasible to obtain, by a minimum of three sets of analyses, some insight into the nature and distribution of the mineral ingredients of the brain. I enumerate the elements which enter into their composition:

Metals: sodium, potassium (ammonium), calcium, magnesium, iron, manganese, copper.

Metalloids: chlorine, sulphur, phosphorus, carbon, oxygen, hydrogen, fluorine.

There is met with at almost all steps of the separation of the ingredients of white matter, a substance distinguished by fusibility and insolubility in boiling alcohol, which was named *stearoconote*, and regarding which a few explanatory notes may properly be given in this place. When white matter is placed into a quantity of hot alcohol, most cholesterin and lecithin and some cerebrin dissolve; but the other phosphorised and nitrogenised matters

immediately fuse into a plaster-like mass, which is then practically insoluble in boiling alcohol. There is therefore here an attraction produced by the fused state which removes the solubility of some of the ingredients of the mass in alcohol. When the principal matters soluble in ether—*i.e.*, the kephalins—are now extracted, the fusibility is diminished, and some of the cerebrins dissolve more freely in hot alcohol. Stearoconote, however, still forms, and is now a reaction mainly of the myelins and the cerebrosides. When all the myelins are separated from the cerebrin group of bodies, their power of forming stearoconote is depressed to a minimum, but not entirely removed. The myelins by themselves also retain this tendency of forming a fused mass insoluble in boiling alcohol, which in the text has been termed a tendency to stearoconotise. The phenomenon seems to be compound, and not simple, and to consist in essence in a dehydration without attendant change in quantities of atoms of other elements. For I have found that some stearoconote free from phosphorus could, by treatment with benzol and a little hydrochloric acid, be transformed into a soluble substance, which on elementary analysis was found to have the same empirical composition as the stearoconote from which it was made. In this case no separation of a base from the substance could be proved to have taken place. In other cases it was found that the stearoconote obtained its solubility in alcohol without the intervention of acids, by standing in benzol, in which it is easily soluble. In a third class of cases the stearoconote regained its solubility in boiling spirit by prolonged treatment with hot or boiling water. Again, in other cases it was probable that the stearoconote was really a compound of some nitrogenised body with a mineral base, and bodies resembling the stearoconotes in some respects, but not in all, were produced by adding bases to solutions of the cerebrin series. On the whole, then, 'stearoconote' does not seem to be a chemical individual differing from the other bodies described, but a function of several bodies, which pass into that state by a molecular change, which change can be made to retrograde into the originally soluble condition.

The function is a result of chemical and physical influences acting simultaneously (alcohol and heat), and is apparently not obtained by either influence separately. Stearoconote seems, therefore, a product, and not an educt; but it is indirectly important, as showing that there is some peculiar attraction or

mutual influence between the bodies of the two great groups. In white matter where those brain-principles are all mixed together nearly in their original proportions (only lecithin being much diminished in quantity), the above influence has its highest intensity. The function diminishes with increased separation—*i.e.*, increased purity of educts. It is entirely lost in some of the educts, when they are in the pure state; thus pure kerasin could by no means be made to yield any stearoconote. But it remains with others, particularly myelin, paramyelin, and sphingomyelin in the pure state, and is evoked when they are subjected to the influence of heat in the presence of a quantity of alcohol insufficient to dissolve the whole of the material before it has had time to fuse. Once fused, it seems to resist solution, even after repeated powdering to increase the surface for the action of alcohol, to the extent of being rather destroyed than dissolved.

In the phenomenon just described we observe as the main result diminished or suspended solubility—that is to say, suspension of the lowest form of chemical attraction. In a phenomenon now to be pointed out, we perceive however, on the contrary, the production or elicitation of a solvent power of a kind hitherto quite unknown in animal chemistry. Some of the phosphorised bodies, when combined with certain metals or metallic salts, in the presence of ethylic ether, and whether dissolved in it or not, cannot be separated from these metals by hydrothion. The dissolved compounds assume the colour of the respective sulphide, and remain dissolved; the compounds insoluble in ether immediately pass into solution when the hydrothion gas is passed through the mixture. Thus, cadmium chloride compounds dissolve with a canary-yellow colour; platinum chloride compounds with a dark brown colour; lead compounds with a red or blackish-red colour. The compounds can be partially or entirely precipitated by water, or alcohol, or ammonia, etc. They contain, in addition to the phosphorised body, in the cases of chlorides, chlorine and sulphur, besides metallic sulphide; in the case of metals like lead, metallic sulphide and sulphur. Their complication places them at present beyond the reach of stoichiometric treatment.

When once thus combined with sulphur (or hydrothion) and metallic sulphide, or with these and chlorine in addition, and precipitated and isolated, the phosphorised bodies can by no means ordinarily at hand be again obtained in the free state. The com-

pounds with metals of the phosphorised bodies can be decomposed by hydrothion only while suspended in water or spirit; the product, a mixture of metallic sulphide with the respective body, must then be extracted by a suitable solvent.

The obstacles which these extraordinary properties of the chemical principles of the brain throw in the way of chemical procedure are perfectly indescribable. To use a simile from military life, the biologist who attacks his problem in front is beaten off at all points; he can only conquer it by flanking on long and circuitous routes, and by the use of instruments of warfare which are either new or superior to those hitherto in use.

These difficulties increase with every step which leads nearer to the consummation of the purpose, and are greatest at the point where the isolated chemical individuals are to be freed from the last traces of admixed impurity. The very peculiarities of the principles described are mostly negations of the properties ordinarily relied upon as criteria of chemical purity. On this matter I have repeatedly dilated in the text, and given expression to apprehensions as well as to considerations calculated to remove them. As the difficulties arose they were followed out experimentally, one by one, step by step, to the exhaustion of the known means at hand, or of the new means that could be devised in the time. But it must be left to the future to increase, if possible, both the means of producing, and also the criteria for insuring, that absolute purity of ultimate educts which is the condition of certainty in chemical science.

It is therefore not asserted that the absolute limits of the subject have anywhere been reached; but it is confidently believed that its entirety has been explored in such a manner that fundamental truths cannot have escaped observation, and that what remains to be done is essentially of the character of detail, which, however vast by multiplicity it may become, will not alter the broad outlines which this investigation has led me to state.

While, then, there may be some degree of uncertainty as to the absolute purity of some of the principles involved, there can be none as to their striking individuality; and as regards this, the positive evidence of their peculiar and distinctive qualities is so strong that the fact of their not uniformly answering to certain other criteria is, in my opinion, quite insignificant.

By the researches embodied in this treatise the brain is shown to be the most diversified chemical laboratory of the animal body; it is shown that all other organs, even when the results of their chemical action, be they destined to take a centripetal or centrifugal course, are added to them, are relatively much more simple and very much less specific in their chemical constitution than the organs producing and conducting nerve-power.

II.

METHOD PURSUED FOR THE ISOLATION OF IMMEDIATE PRINCIPLES.

Preparation and Comminution of Braintissue.—Anyone who would proceed to an extensive chemical investigation of the brain should procure, in the perfectly fresh state, and with the perfect freedom from disease which are indispensable, such a supply of material from the human subject as will suffice for his purpose. Human brains are not only relatively the largest in size, but also the richest in specific ingredients, and therefore the most advantageous objects of inquiry. But they are, under ordinary circumstances, difficult to obtain, and therefore for large and more general inquiries ox brains may be used. Five such brains weigh on an average 1,780 g, or eight weigh six pounds. They are washed once and freed from clotted blood. They are next carefully skinned, the arachnoid and pia mater being removed by means of fine anatomical forceps. When the brains are entire they may be skinned in the ordinary anatomical manner, which employs two pairs of forceps, worked simultaneously and antagonistically by both hands of the operator. When, however, the brains are much broken up, each piece must be held in one hand while being freed from membranes with the other. The skinned parts are again rinsed in water, and then placed in water for a short time; next placed upon a sieve to drain, and then submerged in methylated alcohol of 85 per cent. by weight in volume strength, previously purified by distillation over tartaric acid. Great care is necessary to supply a sufficient amount of alcohol, so that the brains may be quickly dehydrated and hardened. For if the alcohol is too dilute, or becomes too dilute by being insufficient in quantity, the brains remain soft and unworkable,

and decompose with a fetid odour. For the same reason all brains, before submersion in alcohol, must be broken up, or sliced into small pieces, so that they can be easily penetrated by the alcohol. This is required even when strong alcohol is used, as this is liable to harden the outer shell merely, and leave the inside of the brain to soften and decompose.

The washing and submersion in water probably remove small quantities of extractives and soluble salts, besides the blood, and must therefore be carried out with care. The solutions so obtained are thrown away, but the alcoholic solutions in which the brains have been hardened are purified from albumen by boiling and filtration, freed from spirit by distillation, and evaporated to the consistence of extracts on the water-bath. They are to be considered as water-extracts, and are mostly free from specific brain substances, and only yield extractives and salts and other matters, which will be described in the relative chapters.

When the brains are well hardened in the alcohol, which frequently requires the repeated removal of the watery spirit and substitution of fresh strong spirit, they are passed through a rotary mincing machine, and the minced portions are again mixed up with strong alcohol. This pulp is now worked through a very fine hair sieve, having 144 meshes to the square inch, and each of the twelve strands of hair crossing the square inch in one direction, being composed of eight single horse hairs; the sieve stands upon a glass-receiver in such a manner that it cannot move, and no matter which passes the sieve can be lost. The trituration on the sieve is effected by a strong circular brush, which is rubbed over the sieve by the hands of a workman. When passed through the sieve the brain is in the state of a very fine pulp or *purrée*, and is ready for extraction. All other modes of comminution which have been recommended are less useful than the foregoing; they are either inefficient or slow and laborious; in particular, trituration in a mortar with a pestle is very inefficient. All methods of comminution which do not reduce the brain to the finest possible pulp, or smooth paste, must be rejected, as from imperfectly comminuted brains the immediate principles are necessarily most imperfectly extracted.

Extraction of Brainpulp and Separation of Albuminous or Insoluble Matter, White Matter, Buttery Matter, Last Oily Matter, and Ultimate Watery Mother-liquor.—The smooth paste of brain-matter is now

mixed with a considerable amount of alcohol of 85 per cent., and heated in a well-tinned large saucepan over a gas lamp by a star-burner or any other heat source, while being stirred with a wooden rod without intermission. When it has reached the temperature of 70° the mixture is removed, and immediately poured on a filtering cloth stretched and tied over the top of a large earthenware jar or pan. The filter is covered with a wooden cover. When the liquid has percolated, the pulp is removed from the cloth with a flat spoon, and again placed in the saucepan, mixed with spirit, heated to 70° while being stirred, and again placed upon the same filter as before. This operation is repeated in all about five times, when the brain-matter is exhausted of all matters which alcohol at that temperature will dissolve. The matter is now tied up in the cloth and pressed in a screw-press. It comes out as a solid, somewhat elastic cake of *albuminous* or *insoluble matter*, of which the analysis and description will be given in a future chapter. In my earlier experiments I arrested the heating at 45°, because it has been so frequently stated that when brain-matters are heated beyond they decompose. But since I know the behaviour of the isolated brain-matters, I have come to consider this statement as unfounded. Moreover, there is a considerable quantity of cerebrin-like matter in brain which is not at all dissolved by spirit at 45°, but is taken up by boiling spirit, as has been shown in Ann. Chem. Med. i., 1879, 258. To extract all this matter it is necessary to boil the albuminous matter with spirit for many hours in a platinum still, with a condenser attached, and to repeat this at least from twelve to fourteen times. But the low heat is certainly convenient, and need not therefore in the earlier extraction be overstepped. For the same reason a saucepan of cast-iron, about three gallons capacity, is preferable to any other vessel of tin or glass. The earthenware jars or pans should be of a capacity of from twelve to fifteen gallons. I have found them most useful, and carried on all operations from the soaking and hardening to the last filtration of the buttery precipitate with their aid.

White Matter.—The alcoholic extracts are all united, and allowed to cool during from twelve to twenty-four hours. In hot weather the cooling must be assisted by placing the jar in a tub and surrounding it with cold water and ice. The extracts during cooling deposit a large amount of *white crystalline and granular*

precipitate, which adheres to the sides and covers the bottom of the vessel, while the alcohol is perfectly clear, though coloured slightly yellow. The whole is filtered through a cloth stretched over a pan, and when the entire precipitate is collected on the cloth, and has been condensed by stirring with a spoon, the cloth is removed, tied up, and placed in the screw-press, and all mother-liquor thus removed. When taken out of the cloth the precipitate presents itself as a hard white cake, which can be broken into pieces, and constitutes the particular white matter of Vauquelin, and will in the text be signalised as *white matter*. When the abbreviations *W.M.* occur in the description of any preparation, they indicate that the preparation has been extracted from this white matter. I shall not describe this white matter any further, nor have I instituted any experiments upon it such as Vauquelin made, because it is evidently a very complicated mixture, containing nearly the whole of the substances to be described as the cerebrosides, stearoconotes, cholesterins, kephalins, myelins, and lecithins, and small quantities of other matters. The processes by which these substances may be extracted will be given lower down, after the description of the treatment of the alcoholic extract has been completed. Here it may yet be stated that the white matter can be preserved in stoppered bottles, in a cool place and protected from light, almost unchanged for a very long time. In contact with absolute alcohol it also remains unchanged, though gradually yielding a yellow extract; but in contact with ether it yields kephalin to the latter, which is quickly oxidised into a red substance having a green fluorescence; this effect seems due to the peroxide of hydrogen produced during the oxidation of the ether. I have therefore limited the use of ether to the most necessary operations, and then cause the substances to pass through these with great despatch, so that this oxidising effect of the ether is as far as possible avoided.

Buttery Matter.—The Alcoholic Filtrate from the White Matter is now placed in a capacious tinned copper, or better platinum still, and a great part of the alcohol is distilled off. When a certain degree of concentration has been obtained, which is determined by experience, the hot liquid is thrown into a pan, and again allowed to cool, assisted if necessary with cold water or ice. It now deposits a second quantity of matter, which is less solid and

more coloured than the first, and after filtration remains on the cloth as a semi-solid plastic substance, to which I have given the name of the *buttery matter.* This can only be freed from mother-liquor by manipulation with a spoon, and must not be pressed too hard, as it is liable to pass through the meshes of the cloth. The buttery matter consists of much cholesterin, lecithin, little myelin, kephaloidin, and some cerebroside, and small quantities of other matters; the substances are therefore qualitatively mainly the same as in the white matter, but they are present in entirely different proportions. The buttery matter also keeps well in a bottle by itself, or in the presence of alcohol, but should also not be kept long in ether.

Last Oily Matter.—The Alcoholic Filtrate from the Buttery is again distilled so long as good spirit passes over and no precipitate ensues in the fluid. When these conditions are exhausted it is placed in a large dish on a water-bath and evaporated. At a certain period *oily drops* make their appearance, which adhere to the sides or float in the fluid, and unite to larger round globular masses. They separate easily while the fluid is hot, but when the fluid cools they swell, become flaky and distributed in the fluid, and cannot be filtered. They are best separated while hot by a separating funnel, to which they adhere, while the fluid sinks down; or they may be collected on a paper filter on a hot funnel. This matter has received in my laboratory the title of the *last oily*, by which it will be signalised in this essay. It consists mainly of phosphorised bodies with little cholesterin, and some peculiar not yet accurately defined matters.

Ultimate Watery Mother-liquor.—The Filtrate from Last Oily constitutes the ultimate *watery mother-liquor* and contains all matters from the brain which are highly soluble in water, such as the salts, the extractives, and soluble immediate principles to be described. This liquid is evaporated on the water-bath to the consistence of a thin extractive, and placed in bottles until further examined as will be described. While the preparation accumulates it is well to keep the extract covered with some absolute alcohol to prevent the formation of mould on its surface.

Process for separating White Matter into its Constituents.—The following process, which for the purpose of abbreviation I will term 'ether process, W.M.,' yields the cerebrins very quickly and directly, and leaves little myelin, some paramyelin, and most

sphingomyelin, with the cerebrins. It also yields kephalin, but much remains in the mother-liquors. The myelin, lecithin, and cholesterin remain in the ultimate mother-liquor, and can be separated only by cadmium or platinum chloride.

The white matter is fully extracted with ether in stoppered bottles, with the precaution of using the same ether for several portions of white matter so as to obtain saturated solutions. All secondary solutions and washings are concentrated by the still. For this purpose French flasks and a platinum condenser are used; the flasks contain spirals of platinum-wire, and pieces of tobacco-pipe tube strung on platinum-wire. This arrangement prevents bumping. The whole of the solutions are now exposed in bottles (stoppered) to a strong freezing-mixture of ice and salt, and the clear coloured ether is quickly siphoned off the dense white deposit. All siphons are of glass tube, with movable caoutchouc joints, and mounted in corks, so that they can be applied to the bottle to be emptied on one side and to the bottle to be filled on the other side, air-tight, and be started either by blowing (with the air-bellows) or by suction at the opposite end. The inner limb of the siphon is so curved as to be near the side of the bottle and easily visible; its end is directed sideways to prevent an upward rush of deposited matter.

This apparatus gives to the operator full power to start and arrest the flow of the ether whenever he finds it desirable, and enables him to regulate the suction-pipe, and so take off the last portions of ether above the deposit, without losing much ether or being molested by it. When the principal mother-liquor is removed, pure ether is thrown upon the deposit, which is again frozen. The deposit is much less soluble in pure ether than in the mixture of dissolved matters, a peculiarity shown by almost all brain-substances. The deposit, when dense, frequently forms a firm cake at the bottom of the bottle, which comes off as a round disk. These '*first deposits by frost from ether extracts of white matter*' are separated into sphingomyelin, myelin, and other matters, as will be described lower down.

When the ether extracts give no further deposits on exposure to renewed freezing-mixtures, they are treated with absolute alcohol until all kephalin is precipitated. If the alcohol be watery, even slightly, say of 80 to 90 per cent. strength, the deposit contains much cholesterin, particularly if the ether

solution is concentrated. Absolute alcohol should therefore be always taken as well as absolute ether for these operations; many other reasons which will appear in the sequel support this desideratum to the extent of making it an absolute condition of perfect success.

The ether-alcohol mother-liquor is now distilled for removal of the ether, and is then while hot treated with a hot solution of lead acetate and ammonia as long as a precipitate is thereby produced. The precipitate, which contains kephalin-lead, myelin-lead, and a few other compounds, is filtered off. The solution is allowed to cool, and deposits more lead salts and cholesterin. The filtered solution is again distilled while carbonic acid is passed through it. By this means the excess of lead is precipitated and the ammonia expelled. To the alcoholic filtrate alcoholic cadmium chloride is now added as long as a precipitate is produced, and afterwards a large excess of the same cadmium chloride solution. The precipitate is filtered off on a cloth pressed hard, and immediately placed in ether. A cadmium salt of a phosphorised matter dissolves, which is not yet very well defined. Cholesterin also dissolves. The greater bulk of the cadmium chloride precipitate remains insoluble in ether, and consists of lecithin, paramyelin, amidomyelin, and sphingomyelin, all combined with the metallic chloride. These compounds can be separated from each other, and the immediate principles can be isolated from the compounds by processes to be described.

Treatment of the Buttery Matter.—The buttery matter is dissolved in hot spirit, and completely precipitated with hot ammoniacal lead acetate. The filtrate is allowed to get cool and deposit more lead salt and cholesterin, to be separated by ether. The united lead salts, etc., after exhaustion by ether, are treated with spirit, etc., for the isolation of their ingredients, as will be described below. From the main alcoholic filtrate the excess of lead and ammonia are removed by a current of carbonic acid and distillation. To the clear filtrate alcoholic cadmium chloride is added in large excess, and the white precipitate is treated just as the precipitate from white matter above described. Ether extracts from it cholesterin and a phosphorised cadmium chloride compound, while the part insoluble in ether is separated by the benzol process into three or four compounds of lecithin, paramyelin, and amidomyelin.

Treatment of the Last Oily Matter.—This is dissolved in hot alcohol and precipitated by lead acetate and ammonia; the precipitates formed in the hot fluid, and after its cooling, are collected and separated into their constituents by ether and spirit, etc., as will be described. The spirit is freed from lead and ammonia by distillation with the aid of a current of carbonic acid. The filtered alcoholic solution is precipitated with an alcoholic solution of cadmium chloride added in large excess, and the cadmium compounds are collected and treated as will be described.

Summary of Immediate Principles in the crude state or Mixtures thereof isolated.—We have thus separated the brain into the following immediate principles or mixtures thereof:

1. First extractives (by soaking alcohol).
2. Insoluble albuminous residue.
3. White matter, containing—
   - (*a*) Kephalin (with varieties and compounds);
   - (*b*) Lecithin (with varieties and compounds);
   - (*c*) Paramyelin (with varieties and compounds);
   - (*d*) Myelin (with varieties and compounds);
   - (*e*) Amidomyelin (with varieties and compounds);
   - (*f*) Cholesterin and phrenosterin;
   - (*g*) Cerebrin mixture, or mixture of cerebrosides, cerebrinacides, cerebrosulphatides, and amidolipotides, containing also sphingomyelin and assurin.
4. Buttery matter, containing—
   - (*a*) Kephaloidin (with varieties and compounds);
   - (*b*) Lecithin (with varieties and compounds);
   - (*c*) Paramyelin (with varieties and compounds);
   - (*d*) Myelin (with varieties and compounds);
   - (*e*) Amidomyelin (with varieties and compounds);
   - (*f*) Sphingomyelin, assurin, cholesterin and phrenosterin;
   - (*g*) Cerebrin mixture (very small amount), amidolipotides.
5. Last oily matter—
   - (*a*) Lecithin;
   - (*b*) Paramyelin;
   - (*c*) Oily lipoid matters, cerebrols.
6. Ultimate watery extracts of brain, containing—
   - (*a*) Alkaloids;
   - (*b*) Amidoacids and imides;
   - (*c*) Carbohydrate;
   - (*d*) Organic acids and salts;
   - (*e*) Inorganic or mineral salts.

Summary and Arrangement in Groups of Immediate Principles.— In the foregoing descriptions of processes I have, for the purpose of brevity, spoken of the crude immediate principles which were isolated as if they consisted of a single body each; but in the summary list just given I have added to most of the names of these compounds an enlarged definition, by which not only *the pure principle,* but also *varieties of it* and *compounds of it* are said to have been isolated in one and the same operation. This is strictly the case, as will be seen, for example, in the account of kephalin hereafter to be given; but it may be at once stated that the pure immediate principle which gives rise to the name constitutes the great bulk of each preparation, that this bulk is relatively greatly increased by the removal during the process of purification of matters in chemical combination with a smaller portion of the immediate principle, and that the varieties are very similar in type to the principal matter, and whenever they cannot be separated absolutely their nature and quantity can be ascertained by very good approximations. Where matters are so similar in properties as kephalin and kephaloidin, a complete separation of the entire quantity of each from the other is not easily effected, but a quantity of each can be obtained pure from the other, and from the results obtained by their chemical study the composition of mixtures in any proportions can be derived. The immediate principles above enumerated may be arranged in the following groups:

Group of Phosphorised Principles or Phosphatides.

Subgroup of Mononitrogenised Monophosphatides (N : P = 1 : 1):

Lecithins;
Kephalins;
Paramyelins;
Myelins.
(Sphingomyelic acid, a product.)

Subgroup of Dinitrogenised Monophosphatides (N : P = 2 : 1):

Amidomyelins;
Amidokephalins;
Sphingomyelins (Apomyelins).

Subgroup of Dinitrogenised Diphosphatide (N : P = 2 : 2):

Assurin.

Subgroup of Nitrogenised Phosphatide-sulphatide:

Cerebrosulphatide, body from group of cerebrinacides, containing probably phosphorus, nitrogen, and sulphur (?).

Subgroup of Nonnitrogenised Monophosphatides:

First acid from buttery matter, lipophosphoric acid.
Second acid from buttery matter, butophosphoric acid.
(Kephalophosphoric acid, a product.)

GROUP OF NITROGENISED NONPHOSPHORISED PRINCIPLES.

Subgroup of Cerebrosides:

Phrenosin;
Kerasin.

Subgroup of Cerebrinacides:

Cerebrinic acid;
Sphaerocerebrin;
Other principles not yet defined.

Subgroup of Cerebrosulphatides:

Body containing sulphur.

Subgroup of Amidolipotides, or nitrogenised fats:

Bregenin;
Krinosin.

Subgroup of Alkaloids:

Hypoxanthin;
Second alkaloid, gladiolin;
Third alkaloid, tennysin.

Subgroup of Amidoacids and Amides:

Leucin and homologous principles;
Tyrosin;
Urea.

GROUP OF PRINCIPLES COMPOSED OF THREE ELEMENTS ONLY.

Subgroup of Alcohols, nonnitrogenised:

Cholesterin;
Phrenosterin (?).

Subgroup of Carbohydrates:

Inosite;
Glycogen (?).

Subgroup of Organic Acids, nonnitrogenised:

Formic acid;
Sarcolactic acid;
Succinic acid;
Oxyglyceric acid.

Group of Albuminous Substances.

Subgroup of Nitrogenised Sulphatide-phosphatides:

Plastin;
Gangliocytin, Cytophosphatide (a Nuclein).

Subgroup of Nitrogenised Sulphatides:

Albumen;
Collagen.

Group of Inorganic Principles, including both acids and bases, and salts, either free or in combination with many of the foregoing organic principles. This comprises:

Sulphuric acid;
Hydrochloric acid, and chlorine in chlorides;
Phosphoric acid;
Carbonic acid;

Potash Soda Ammonia Lime Magnesia Copper Iron Manganese	In combination with immediate principles, forming their bases, or in combination with phosphoric acid, and attached to immediate principles as phosphates, or in combination with mineral acids, as free mineral salts in the juices and extracts.

Alumina, silica, fluorine (doubtful).

III.

GROUP OF PHOSPHORISED PRINCIPLES OR PHOSPHATIDES.

General Properties of the Phosphorised Principles.—This group comprises kephalin, kephaloidin, myelin, sphingomyelin, assurin, lecithin, paramyelin, amidomyelin, and their congeners and compounds. All these bodies have certain properties in common, which point to some similarity in chemical constitution; by other peculiarities, again, they are sharply distinguished from each other. *Some are soluble in water in a certain manner and measure.* When they are in the dry state, and are placed in pure water, they sink to the bottom, and are at once wetted by the water. Thus their specific gravity is shown to be greater than that of water, and by this, and the faculty of being wetted, they are sharply distinguished from the fats or fatty acids, but assimilated to the soaps, as the older authors correctly stated. When they have remained in the water for a short time they begin to swell, to become transparent at the thin margins of the particles, and covered with a loose layer all over their surface. This on agitation is easily detached, and floats in clouds in the liquid; the clouds diffuse themselves indefinitely throughout the whole of the water, and if enough water is present, the mixture is frequently shaken, and some time is given, the particles of solid matter disappear entirely and form a turbid solution of very peculiar appearance. As will be seen hereafter in particular, these solutions can by no mechanical means be clarified; yet they are solutions, and pass through many layers of the finest Swedish filtering-paper. Most particles are so small as to be beyond the reach of optical definition as single particles. They exhibit their presence, however, by iridescence in the case of myelin and lecithin, and by

reflecting polarised light in all cases. These solutions, therefore, resemble somewhat the cold solutions of soaps, and the emulsions produced by solid fatty acids with neutral phosphates, the emulsion produced by vegetable seeds (almonds) with water, etc. ; but they differ from milk and emulsion of fats and gum by there being few particles visible. Moreover, emulsions are supposed to require the presence of two agents besides water, whereas these brain substances give this peculiar solution with mere water ; they are not decomposed, as the soaps are, into acid and alkaline salt, as they are not salts, but form these solutions in the free and uncombined, but also in the combined state. I can give no better definition of this peculiar condition than by describing it as *a state of imperfect or incomplete solution*, a stage intermediate between the solid and the fluid state of matter. Those who do not coincide in this description may term the solution an 'emulsion,' if that conveys any definite idea, or a state of the finest subdivision of particles, with peculiar attraction of these particles to water, and consequent repulsion of particles from each other. This latter part of the question I shall have to consider at greater length when I come to discuss the dependence of the structure of the brain upon the chemical and physical characters of its ingredients. For the present purpose it suffices to sum up that these phosphorised bodies have all an extreme attraction for water, swell in it, and ultimately form a nearly perfect solution. Amidomyelin, when obtained by dialysis from cadmium chloride salt, forms a perfect solution, which filters like water, but congeals by warmth. Sphingomyelin, when quite pure, contracts again and then sinks in water, and can be filtered out of it. By this means they afford good opportunities for mechanical and chemical purification now to be described.

Deposition of Impurities.—The solutions, on standing, deposit any mechanical impurities, and any cholesterin contained in the matters dissolving crystallises out and sinks to the bottom. Any excess of the matters beyond saturation is also deposited, as well as the less soluble compounds—*e.g.*, sphingomyelin. If any cerebrin were with the matters, it would also deposit. All solutions, therefore, produced as above, and which experience has shown me should not contain above 1 per cent. of the matters, must be allowed to stand for a day or two, in order that these insoluble impurities and admixtures may be deposited. The pure solution

is then removed with the siphon from the deposit, the solution is filtered, and the deposit further exhausted with water.

Filtration of the Watery Solutions of the Phosphorised Matters.—When the solutions are placed on an ordinary filter of paper, a portion passes by gravitation; but the pores of the paper gradually become obstructed, and filtration ceases. It is therefore necessary to expedite filtration by the aid of pressure. For this purpose I have constructed a new apparatus, in which a vacuum draws the liquid to be filtered into a hollow cylinder surrounded with paper.

The filter is a hollow cylinder of silver-plate pierced like a fine sieve, and covered with a six-fold roll of Swedish or Rhenish filtering-paper, made secure at the top and bottom by a ring of string, which also runs spirally over the whole paper. This stands in a glass cylinder, which contains the fluid to be filtered, and is always kept full by a perpetual siphon, drawing the liquid from the reserve bottle; as frequently as the fluid in the wide upper part of the cylinder sinks below the oblique end of the air-tube, which is wide and provided with a bulb, air is admitted, and the siphon acts. The bottle receiving the filtrate is evacuated of air gradually by means of the air-pump. The tube which draws the filtered liquid from the cavity of the cylinder goes to the bottom of the cylinder, so that nearly the whole of the filtered liquid can at any time be drawn into the receiver.

Such a cylinder, about a foot long and four inches in circumference, will, with a pressure of 70 centimetres of mercury, filter a Winchester quart full of 1 per cent. solution of phosphorised matter per day: the more, the purer the substance is; the less, the more cholesterin it contains. Sometimes only 100 cc. are filtered per hour. In such a case a coarser filtering-paper should be rolled over the Swedish, to collect the coarsest particles and prevent them from getting upon the Swedish paper. After some hours filtration becomes slow, and ultimately ceases entirely; then the paper is found covered with a gelatinous mass of undissolved matter and impurities. These may be rinsed off and again extracted and filtered, but I have mostly found the matter so small in quantity that I have discharged it with the paper. Such a filter, with two changes of paper per day, may be going night and day, and, if all corks are air-tight, which is easily effected by

applying hot paraffin to them, requires very little attention except a few strokes of the pump from time to time. When the substances previous to their solution in water were very pure, they left no vestige of cholesterin crystals or of cerebrosides on the filter. The filtered fluids are still opaque, those from kephalin more coloured, those from myelin and lecithin of a milky whiteness with the blue iridescence. When a good filtration had been effected, a second filtration effected no improvement, and was quickly accomplished. In this manner all phosphorised matters used for cardinal preparations were passed through the process of watery solution and filtration.

The process offers peculiar advantages for each of the three varieties of phosphorised principles. Kephalin scarcely ever retains any cholesterin, but myelin always does, unless it has passed through the $PtCl_4$, or lead process.

Solubility of Phosphorised Matters in Ether.—Kephalin dissolves in this reagent in almost any quantity; lecithin only less than kephalin; but myelin is very little soluble in cold ether, more in hot ether, and is instantaneously deposited from the ether as it cools. Sphingomyelin behaves in a similar manner.

Solubility of Phosphorised Matters in Alcohol.—Lecithin dissolves in all proportions in hot absolute alcohol, less in cold; kephalin is almost insoluble in cold alcohol, more soluble in hot, almost entirely deposited on cooling; myelin, paramyelin, amidomyelin, and sphingomyelin are, however, little soluble in cold alcohol, largely soluble in hot, and deposited on cooling in a crystallised state, and in such quantities that the fluid becomes filled with crystals. It will thus be seen that, while water offers no means for the separation of these substances from each other, ether and alcohol offer great advantages, which have indeed been utilised in the construction of the method for their separation above described.

A. SUBGROUP OF MONONITROGENISED MONOPHOSPHATIDES. N : P=1 : 1.

LECITHINS.

Definition.—Lecithins are phosphorised and nitrogenised immediate principles of brain-tissue and egg-yelks. There are three varieties known, all containing neuryl, oleyl, glyceryl, and a third

acid radicle replacing hydroxyl in the phosphoric acid; the three varieties differ as regards the third acid radicle.

Isolation.—Lecithins can be isolated by the following process: The alcoholic extract of brain-matter from which white matter has been deposited is concentrated until on cooling it forms the deposit called *buttery.* This is isolated by filtration, re-dissolved in warm spirit and treated with lead acetate and ammonia as long as a precipitate is produced, and filtered hot. The filtrate deposits a crystalline mixture of cholesterin, lead salts, and lecithin. This mixture is again isolated by filtration, dissolved in hot spirit, and allowed to crystallise again. To the solution filtered from the first deposit as well as to that filtered from the second crystals an alcoholic solution of cadmium chloride is now added, as long as a precipitate is produced; then half the volume of the cadmium chloride solution already used is added to the mixture. The precipitate is isolated by filtration and dissolved in boiling spirit; the solution, filtered hot, is allowed to deposit the salt, which is then filtered off. This is a mixture of three, maybe four, compounds, one of lecithin with cadmium chloride, another of paramyelin with the same salt, a third of amidomyelin, and a fourth of sphingomyelin with cadmium chloride. The last is, if at all, present in the smallest quantity; the lecithin and amidomyelin compounds are present in about equal quantities, and constitute the great bulk of the precipitate. The precipitate is dried in vacuo over sulphuric acid (when dried on the water-bath it becomes coloured), finely powdered and exhausted with ether. It is again dried and powdered, and treated for the separation of the several compounds as follows:

The dry precipitate is suspended in a large volume of anhydrous benzol and frequently stirred; kephalin cadmium chloride and kephaloidin cadmium chloride are extracted at this stage; the mixture is then boiled for some time in a water-bath, and a portion of the benzol is removed by distillation. The mixture is now allowed to stand in a cold place for twenty-four hours, and then thrown on a filter. The matter remaining on the filter is subjected to the same treatment with fresh benzol as often as may be necessary to exhaust it of all matter soluble in cold benzol. The matter insoluble in cold benzol is now again boiled with benzol and thrown on a filter kept hot by a steam-jacket. A benzol solution now passes through the filter, which deposits a

compound on cooling, and keeps little or nothing in solution. This treatment also is repeated until the insoluble compound yields nothing to boiling benzol. The cadmium chloride precipitate has thus been separated (leaving the kephalin compounds out of consideration for the present) into three different matters :

(1) Compound soluble in cold benzol, lecithin cadmium chloride.
(2) Compound soluble in hot benzol, deposited on cooling, paramyelin cadmium chloride.
(3) Compound insoluble in benzol, cold or boiling, amidomyelin dicadmium chloride.

As sphingomyelin is somewhat soluble in cold alcohol, it is to be expected that a small quantity is present in the mixture of which the three compounds just defined are the principal constituents. It may be separated from the amidomyelin in the manner to be described below. But its presence in the cadmium chloride precipitate from buttery matter has not been proved ; in the process of separating the educts by solvents only it remains mainly if not entirely with the cerebrosides and cerebrinacides, after the lead process with the cerebrosides only, to one of which, kerasin, it has a slight chemical affinity. This might explain its absence from the buttery matter.

Episode concerning the Shifting of Cadmium Chloride in Mixtures of Lecithin, Paramyelin, and Amidomyelin during recrystallisation from Spirit.—It was observed that precipitates which had been exhausted with ether, and therefore were free from uncombined lecithin, after recrystallisation from spirit left some free lecithin in the mother-liquor, which could be precipitated by renewed addition of cadmium chloride solution. It had also been observed that such precipitates containing amidomyelin showed a higher percentage of cadmium chloride than before. Similar data were also obtained during the treatment and analyses of the platinum salts. On the basis of these observations the following hypothesis was formed.

Amidomyelin probably combines with cadmium chloride in two proportions, namely, either with one molecle of this salt, or with two molecles, thus :

$C_{44}H_{92}N_2PO_{10} + CdCl_2$, containing 17·90 per cent. $CdCl_2$.
$C_{44}H_{92}N_2PO_{10} + 2CdCl_2$, containing 30·37 per cent. $CdCl_2$.

The former has a tendency to saturate itself in the presence of lecithin cadmium chloride, $C_{42}H_{84}NPO_9 + CdCl_2$, containing 19·42 per cent. $CdCl_2$, and during recrystallisation from spirit, or during suspension in benzol in which the lecithin salt is in solution, takes up some cadmium chloride from a portion of the lecithin, which is thereby set free and remains in the spirituous, or as the case may be, benzol mother-liquor. This kind of shifting of the cadmium chloride is more likely to occur in anhydrous solvents, or strong spirit, whereas the dissociation under the influence of water, complete during the process of dialysis, is partial, particularly in dilute solvents or edulcorants.

Continuation of the Description of the Process.—The solutions in cold benzol obtained in the process described in the foregoing are allowed to stand for some days in the cold and repeatedly filtered. They are then concentrated, allowed to stand, and filtered again, until the residual solution remains quite clear and free from deposit. Filtration is absolutely required to prove the absence of deposits, as these are so transparent that they easily escape from observation by the eye.

The concentrated thick benzol solution, which is mostly coloured and strongly fluorescent, may now be evaporated to dryness, and the residue further studied. But it is preferable to add to it absolute alcohol as long as a precipitate ensues. Time is again wanted to complete the precipitation. The precipitate is washed with alcohol, dried in vacuo, exhausted with ether, and recrystallised from spirit. Now a portion remains insoluble in boiling spirit, but the bulk dissolves and is deposited on cooling as a white mass, which, under the microscope, is seen to consist entirely of needles arranged radially in stars and balls.

Separation of Lecithin from Amidomyelin and Paramyelin when all are in the free state.—All three bodies are precipitated by cadmium chloride, and the compounds are soluble in boiling spirit. When the mixture is decomposed in one way or another, and the resulting free bodies are dissolved in hot spirit, the amidomyelin and paramyelin separate first in the shape of white leaflets, again on concentration ; from the highly concentrated spirit a mixture of lecithin and little amido- or paramyelin is at last deposited as an unctuous mass. When this is treated with ether, white amido- or paramyelin remains insoluble. When the ether solution is distilled to dryness and the residue treated with *absolute* alcohol,

lecithin dissolves, while again some amido- or paramyelin remains insoluble. The absolute alcohol solution deposits more amido- or paramyelin on long standing, particularly at low temperatures. But it is, perhaps, not practicable to obtain perfectly pure lecithin without a trace of amido- or paramyelin by this process, although the amido- or paramyelin obtained by it is perfectly free from lecithin. A specimen of lecithin thus prepared (by dialysis, etc., but not separated as $CdCl_2$ salt by benzol) gave on analysis 3·66 per cent. P. and 2·83 per cent. N. The amount of nitrogen shows that the body contained yet some amidomyelin.

The lecithin, when thus purified as far as possible, gives a white $CdCl_2$ salt which is soluble in boiling spirit, and when deposited from it leaves some coloured impurity in solution.

On the mode of separating lecithin from amidomyelin by benzol, when both bodies are combined with $CdCl_2$, *see ante*, and in the relative paragraph under amidomyelin. Paramyelin can be separated to some extent from amidomyelin by the greater solubility of its hydrochlorate, but completely only by the solubility of its cadmium chloride compound in boiling benzol, in which amidomyelin is insoluble.

Properties of Lecithin.—Lecithin is a white crystalline body, crystallising in thin plates, which when compressed form a wax-like mass. It is very soluble in spirit, being only deposited when the solution is extremely concentrated. A slight rise in the temperature causes the crystals to redissolve. When to its cold saturated solution in spirit water is gradually added until a permanent considerable turbidity is produced, and when this turbidity is cleared up by heating, the solution deposits on standing lecithin in the semi-solid hydrated state. The solution in 80 per cent. spirit treated as described left 3·08 per cent. lecithin on evaporation. The unctuous lecithin under the microscope consists of balls in concentric layers. Lecithin is easily soluble in ether.

It is easily soluble in chloroform, and is left on evaporation as a non-crystalline mass.

It dissolves in oil of vitriol with a yellow colour, and on addition of thick cane-sugar syrup Raspail's reaction, a purple colour, is gradually produced, changing slowly into black. The reaction is due to the oleyl radicle contained in the lecithin.

Compounds of Lecithin.—An alcoholic solution of lecithin is precipitated by *cadmium chloride;* the white voluminous precipitate,

amorphous at first, crystallising on standing in the mother-liquor, is easily soluble in boiling spirit, and deposited on rapid cooling in white crystalline granules; on slow cooling, acicular crystals arranged in balls and rosettes are deposited. They are very uniform and characteristic, and totally different from sphingomyelin cadmium chloride. This precipitate is insoluble in cold and boiling ether, easily soluble in benzol.

Platinum Chloride Hydrochlorate of Lecithin.—Lecithin is precipitated by an acidified solution of platinum chloride in spirit; the voluminous yellow precipitate is easily soluble in ether, and again precipitated from this solution by absolute alcohol. Its formula is $2(C_{43}H_{84}NPO_8) + 2HCl + PtCl_4$. A compound also occurs with one HCl only, and another without any HCl, and containing $PtCl_4$ only.

Lecithin is not precipitated by a mixture of lead acetate and ammonia.

Solubility of Lecithin $CdCl_2$ Salt in Spirit.—Ten cc. of the 84 per cent. spirit solution which had deposited a crystallised salt, on evaporation left 0·0387 g. solid matter. One part of the salt therefore requires 258 parts of spirit at 17° C. for solution.

Bearing of the Lecithin $CdCl_2$ Salt with Benzol.—When the crystallised salt is placed in pure dry benzol it swells and becomes transparent, but does not dissolve. If now the mixture be heated for some time, the salt dissolves without residue, and the clear colourless solution does not form any deposit on cooling and standing. This phenomenon is due to hydration of the crystallised salt; during the first boiling with benzol the water is evolved and passes over with the benzol vapours; when clear benzol passes over, all salt is and remains in solution.

Lecithin Hydrochlorate, $C_{43}H_{84}NPO_8 + HCl$.—The pure white cadmium chloride compound is suspended in spirit, and the mixture is saturated cold with hydrothion. It is now heated in a water-bath to boiling while the current of the sulphuretted gas is continued, until the filtrate is no longer affected by it. The decomposed matter is thrown on a filter arranged on a steam-jacket, and the colourless spirit solution of lecithin hydrochlorate is separated from the cadmium sulphide. The solution deposits the hydrochlorate as a felted mass of crystals. By slow crystallisation from dilute solutions white crystals are obtained. As the cadmium chloride yields two molecles of hydrochloric acid, of

which only one combines with the lecithin, the mother-liquor contains free acid in solution. As this acid decomposes lecithin at high temperatures, it is not advisable to endeavour to obtain the lecithin hydrochlorate which remains in solution in the spirit by evaporation of the latter by heat. It is preferable to extract all hydrochloric acid from the solution by mercuramin, reprecipitate the lecithin by cadmium chloride, and redecompose this by hydrothion in a minimum of spirit. If it is desired to obtain the whole amount of lecithin in a given preparation as hydrochlorate, the body should be neutralised with the acid, and the solution evaporated in a vacuum over lime and oil of vitriol.

The hydrochlorate crystallises in thin leaflets, to be seen by the microscope; they are hexagonal, frequently saucer-shaped, and, owing to their extreme tenuity, mostly so distorted and crumpled up that they appear as a confused mass of curved needles.

(*Human*) *Lecithin Hydrochlorate.*—By $CdCl_2$ from alcoholic solution of white matter and buttery. Passed through benzol process; the soluble in the cold part again recrystallised from spirit. The white salt was decomposed by hydrothion, the solution allowed to crystallise; the crystallised mass was recrystallised. Dried in vacuo to a perfectly white mass which could be powdered easily. At 98° it became a little soft, and by prolonged heating somewhat coloured. It gave on analysis 4·84 per cent. HCl, 2·03 per cent. N, and 4·29 per cent. P; therefore, HCl : N : P = 1 : 1 : 1.

(*Ox*) *Lecithin Cadmium Chloride.*

Theory.			Found.	
Atoms.		Per cents.		
43 C	516	53·9	—	—
84 H	84	1	—	—
1 N	14	1·46	1·34	—
1 P	31	3·24	—	3·28
8 O	128	—	—	—
1 Cd	112	11·71	—	—
2 Cl	71	7·42	—	—
	956			
Lecithin	773	80·87		
Cd Cl_2	183	19·13		
	956			

$C_{42}H_{82}NPO_8 + CdCl_2$, At. W. = 942, requires 19·42 per cent. $CdCl_2$.

CHEMOLYSIS OF LECITHIN.—Lecithin, when isolated as platinic chloride hydrochlorate salt, immediately after isolation begins to decompose, and completes this decomposition during the steps necessary for its purification. Its platinum salt, which is in the first instance soluble in ether, becomes in the air or in the vacuum speedily covered with oily drops (oleic acid), and the residual salt is then insoluble in ether. It was upon such changed platinic salt insoluble in ether that the following chemolysis was effected:

Water alone was found capable of removing at least some of the platinic chloride; but this treatment was not persisted in, and the salt was boiled with two molecles of BaH_2O_2 during two hours. The decomposition was effected very readily, a black precipitate (of platinum) being formed simultaneously and insoluble barium salts of certain fatty acids.

The Barium Salts.—These were washed with water, and then decomposed by hydrochloric acid, in the presence of ether. The ether solution was distilled to dryness, and the residual matter converted into ammonium soap; and the latter in its turn was converted into barium salt once more, and after drying extracted with much boiling absolute alcohol. The alcoholic extraction was continued to perfection. The extracts deposited a white salt on cooling, which was isolated, dried, and analysed; it gave 19·55 per cent. Ba. Oleate of barium requires 19·59 per cent. Ba. This salt was therefore *oleate of barium.*

The barium salt left undissolved by the alcohol was insoluble in ether. It was once more decomposed by hydrochloric acid in the presence of ether, and converted first into ammonium soap, and again into barium salt. It was now isolated, dried at 100° C., and analysed; it gave 19·82 per cent. Ba, and 57·76 per cent. C and 9·86 per cent. H.

Computation of Analyses:

	Percentages.	÷At. Wgts.	Ba=1.
C	57·76	4·813	33·4
H	9·86	9·86	68·4
Ba	19·82	·144	1·0
O	12·56	·785	5·4

$$=C_{34}H_{66}BaO_4.$$

This was, therefore, apparently *margarate of barium.*

The Barita Solution.—The excess of barita was first removed from the solution by means of carbonic acid, and then nitric acid

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was added to strong acidity. In this state the solution was precipitated by phosphomolybdic acid, and the filtrate reserved. The precipitate was decomposed by hot barita and concentrated to a low bulk. The barium which still remained in combination with the base was carefully removed by the exact amount of sulphuric acid necessary, and after concentration the resulting solution was neutralised by HCl and precipitated by alcoholic $PtCl_4$. The platinic compound crystallised from water in long prismatic needles.

The quantity of the platinum salt obtained was very near to that which should have been obtained if all the nitrogen had existed in one form in lecithin, and had been obtained in one form.

Computation of Analyses:

	Percentages.	÷At. Wgts.	÷Pt – 1.
C	18·92	1·576	9·78
H	4·68	4·68	29·00
N	4·63	·330	2·00
O	5·61	·350	2·10
Pt	31·79	·161	1·00
Cl	34·37	·968	6·00

$$= (C_5H_{13}NO)_2\,(HCl)_2\,PtCl_4.$$

The filtrate separated from the phosphomolybdic acid precipitate of the base was found to contain glycerophosphoric acid. The chemolysis of lecithin, therefore, is complete, as there were obtained:

Oleic acid - - -	=	$C_{18}H_{34}O_2$
Margaric acid - -	=	$C_{17}H_{34}O_2$
Glycerophosphoric acid	=	$C_3H_9PO_6$
Neurin - - - -	=	$C_5H_{13}NO$
Total - - -	=	$C_{43}H_{90}NPO_{11}$
Deduct - -	=	H_6O_3 entered in chemolysis
Leaves - -	=	$C_{43}H_{84}NPO_8$ = lecithin.

I have lately chemolysed a specimen of lecithin which had been as $CdCl_2$ salt entirely soluble in cold benzol, and had been crystallised from spirit, after several earlier crystallisations had been removed. It yielded oleic acid, margaric acid, glycerophosphoric acid, and neurin. In the course of this chemolysis I observed that benzol is a better medium than ether for separating

the lead oleate from the lead margarate. This lead process also yields a purer margarate than the exhaustion with alcohol adopted above; but the oleate is again less pure than that deposited from alcohol.

Note on Oleic Acid and its Reaction with Oil of Vitriol and Sugar.—Oleic acid is generally considered to have much affinity for oxigen, so as greatly to impede attempts to obtain it in a pure state. This absorption of oxigen is considered and stated to be attended with the production of a brown colour, and a change in the nature and properties of the acid.

Pure oleic acid, prepared expressly from oil of almonds, with the view of comparing it with the oleic acid furnished by lecithin, showed none of these properties. The potassium, ammonium, lead, and barium salts were all white, and showed no tendency to become coloured on exposure. The free acid was also free from this tendency, and was only very faintly yellow, while its solution in ether was colourless and did not become brown on exposure. To prove the purity of the body the barium salt was analysed for barium, and found to contain 19·73 per cent. as against 19·60 per cent. required by theory.

Sulphuric acid turned oleic acid yellowish and red, but chloroform had no action on the mixture. It gave a solution with acetic acid which was so turbid that no spectrum could be obtained.

But with sulphuric acid and sugar it immediately turned purple (with sulphuric acid alone it was yellowish-brown), and the product dissolved with a splendid purple colour in acetic acid; the solution presented the following spectrum:

In the concentrated state it passed red at A, thence a shade increased to D, then black. A more diluted solution presented one broad absorption band between D and E. End at G.

The edges of this band shaded off so very gradually that it was difficult to fix the margins; this solution was red, but presented a green fluorescence; it was not brilliant, and became more turbid on standing.

Oleic acid in chloroform was now mixed with a drop of sugar syrup, and then with sulphuric acid; it immediately became intensely yellow; on stirring and breathing upon it, it became purple, but the colour was very dark and mixed with brown.

It was soluble in glacial acetic acid, giving the above spectrum. Chloroform extracted a splendidly purple matter, leaving a dingy

one behind. This solution, suitably diluted and kept anhydrous by sulphuric acid, passed red to C. Further diluted, there was a band between C and D, and another between D and G; blue to C. Again diluted, band 1 disappeared and band 2 contracted. Acetic acid solution of same test gave same band. The band became shaded off near green. Therefore with chloroform it presented the same spectrum as phrenosin and kerasin, but these were insoluble in acetic acid, in which the oleic acid test was soluble.

Theory of Lecithins considered as Phosphatides.

Oleo-Palmito-Glycero-Neuro-Phosphatide.

$$OP\left\{\begin{matrix} C_{18}H_{33}O_2 \\ C_{16}H_{31}O_2 \\ C_3H_7O_3 \\ C_5H_{11}N \end{matrix}\right\} = C_{42}H_{82}NPO_8.$$

Oleo-Margaro-Glycero-Neuro-Phosphatide.

$$OP\left\{\begin{matrix} C_{18}H_{33}O_2 \\ C_{17}H_{33}O_2 \\ C_3H_7O_3 \\ C_5H_{11}N \end{matrix}\right\} = C_{43}H_{84}NPO_8.$$

Oleo-Stearo-Glycero-Neuro-Phosphatide.

$$OP\left\{\begin{matrix} C_{18}H_{33}O_2 \\ C_{18}H_{35}O_2 \\ C_1H_7O_1 \\ C_5H_{11}N \end{matrix}\right\} = C_{44}H_{86}NPO_8.$$

C	42	504		43	516	44	528
H	82	82		84	84	86	86
N	1	14		1	14	1	14
P	1	31	4·08	1	31	1	31
O	8	128		8	128	8	128
		759			773		787

The first three radicles in each formula replace a molecle of hydroxyl each; but the addition of neurin takes place under circumstances which engender the expulsion of a molecle of water from the neurin itself. The mobility of this molecle of water in neurin is clearly shown in the course of the synthesis of this base.

2. Kephalins.

a. Kephalin.

Purification.—The crude kephalin obtained by the primary operations is dried under the air-pump over sulphuric acid. It

must be repeatedly taken out and flattened out in a mortar, and again dried before it becomes dry and brittle, and can be powdered. Resolution in absolute ether, reprecipitation by absolute alcohol, and redrying causes a great improvement, but also much loss.

The dry substance is then dissolved in pure water, 10 g. in the litre, and after complete disintegration by agitation in a stoppered bottle, is allowed to deposit less soluble salts and impurities, decanted or siphoned from these, and then filtered by pressure as described.

The filtered solution is now treated with enough hydrochloric acid to effect complete precipitation of all kephalin as hydrochlorate, a salt which appears in voluminous flakes, and on shaking collects on the surface of the liquid. (This precipitation, like others to be related, affords a good criterion of the previously dissolved state of the substance.) The mother-liquor is drawn from underneath the precipitate with a siphon ; fresh water is poured on the precipitate, and the whole is now placed upon a paper filter or cloth, and washed with water until it begins to swell and dissolve. At that point it is found that all HCl is washed out, and that only *pure hydrated kephalin* remains on the filter. This was expressly proved by analysis in several cases, but more especially in the following experiment.

Expulsion of Hydrochloric Acid from Kephalin by Water.—Half a litre of a weak kephalin solution was precipitated by HCl and filtered ; the precipitate on filter was placed in 500 cc. of water to test its solubility, and was found insoluble, but the water contained free HCl. It was again filtered, and after the solution had run through absolute alcohol was blown on the precipitate; this became adhesive, and the solution refused to filter. The alcohol and precipitate were transferred to a beaker, and the whole was gently warmed in a water-bath to a temperature not much above 60°. The precipitate fused, but dissolved very sparingly, apparently the less the more the alcohol made the precipitate anhydrous. The alcoholic solution was now filtered off warm, and the undissolved portion of the precipitate taken up with ether. The solution so obtained was of a colour resembling ordinary kephalin in ether, and was poured into the alcoholic solution. The precipitate which ensued was fawn-coloured, adhesive, and leathery ; it was isolated, washed with absolute

alcohol, the mother-liquor being squeezed out of it, then pressed and dried in air-pump. The whole was analysed for chlorine as follows. It was heated to dryness in a strong solution of pure soda, with carbonate and nitre added, and the mass was burned. The fused mass was dissolved in dilute nitric acid, and the solution tested with silver nitrate. No precipitate occurred, showing that *the body obtained in this process is not a hydrochlorate*, but kephalin in a purified state.

Bases and Salts which are in combination with Kephalin after Filtration of its Aqueous Solution.—The solution of hydrochloric acid and other matters filtered from the precipitated kephalin was evaporated to dryness. A portion was then boiled with solution of barita, when traces of ammonia were evolved. The rest of the residue was then ignited in a platinum dish to destroy all traces of organic matter. The ash was slightly molten, and only partially soluble in water, but easily soluble in water slightly acidified with hydrochloric acid. The solution was filtered from a trace of carbon, and treated with excess of ammonia, whereupon an abundant precipitate of *earthy salts* fell down, and the solution assumed a *deep blue colour*. Precipitate and solution were separated by filtration. The precipitate dissolved readily in a little HCl, forming a slightly red solution, indicating presence of *iron*, which was confirmed by the sulphocyanide test. In another portion of the solution dilute sulphuric acid gave an abundant precipitate of gypsum, showing presence of *calcium*. In another portion the molybdate test showed the presence of *phosphoric acid*. To the remaining portion a few drops of ferric chloride were added, then sodic carbonate nearly to neutrality, and lastly, excess of baritic carbonate. This mixture was allowed to stand, filtered, and the filtrate, freed from excess of barita by sulphuric acid, was again filtered. The filtrate, after supersaturation with ammonia, gave a great precipitate with ammonium oxalate, insoluble in acetic acid, showing presence of much calcium. The filtrate from this calcic oxalate on concentration and treatment with ammonia, ammonium chloride, and sodium phosphate, gave the precipitate characteristic of *magnesium*. The precipitate produced by barium carbonate was boiled with excess of pure soda, and the filtrate warmed with ammonium chloride, when only a turbidity was produced, indicating the *absence of aluminium*.

The alkaline filtrate from the foregoing precipitate by ammonia

was tested for lime by oxalate, when a considerable precipitate was produced, showing the *presence of lime uncombined with phosphoric acid*, and which must therefore have been in combination with part of the kephalin. The blue solution was again filtered from calcium oxalate, which had been entirely precipitated, and acidified with hydrochloric acid. The *copper* was precipitated by hydrothion, the filtrate evaporated to dryness, ignited, and the residue tested for alkalies. This residue was considerable, surmounting in quantity or bulk the bases previously removed. It was fusible with ease, and on solidification became white and crystalline, but interspersed with many red particles of ferric oxide. The fusion showed it to be mainly *potassium chloride*, but there was also some *sodium chloride* present, as indicated by the flame reaction, and the ferric oxide which had escaped precipitation by excess of ammonia.

It is therefore proved that the kephalin obtained by the alcohol and ether processes, and purification by solution in water and filtration, consists of kephalin in the free state; and of kephalin combined with ammonium, sodium, potassium, calcium, iron, copper, and with calcium and magnesium phosphates.

This experience was repeated a great number of times on, in the aggregate, several hundred grammes of dry kephalin; the calcium and potassium salts were always found prevailing greatly in quantity over the others; none were ever absent. Although I have not compared the total of the neutralising power of the bases with the total of the acid-combining power of the kephalin, by direct quantitative experiment, I am sure that much kephalin must have been present in the free state, as will appear from future developments concerning the combining and dissociating powers of this body.

Dialysis of Kephalin.—A solution of 5 g. of crude kephalin was dissolved in 500 cc. of water, and formed a white thick liquid, from which some cholesterin crystallised on standing. It was then placed on a dialyser of parchment paper. A trace of kephalin passed into the water, but so small was the quantity that no chemical operation could be undertaken with it.

Kephalin does, practically, not dialyse, but acts as a colloid, and allows its impurities to pass out into the water.

Clarification and Decolorisation of Watery and Ethereal Solutions of Kephalin.—To a solution of 1 g. kephalin in 100 cc. of water,

5 cc. of filtered fresh *white of egg* were added, and the mixture was heated in a flask in the water-bath; it remained turbid, and no separation of coagulated albumin took place. The mixture had a faintly alkaline reaction. On addition of a drop of acetic acid to the heated mixture a copious precipitate ensued, which enclosed both kephalin and albumen (the kephalin solution by itself, cold, is only partially or imperfectly precipitated by the same acetic acid). By filtration a perfectly clear liquid was obtained, which was no longer precipitated by barita water or platinic chloride, and not changed by boiling. Consequently, all albumen and all kephalin were removed together from the solution, and perhaps in part combined. Cold absolute alcohol in large quantity extracted all or nearly all kephalin from the albumen, and on distillation left it *perfectly white.* The albumen, on the other hand, was, after washing with alcohol and ether, in a finely divided pulverulent state, and not hard nor horny. This process is therefore useful for preparing snow-white kephalin, which must, however, not again be brought into contact with ether, as that would immediately cause it to become coloured under the influence of oxidation.

Influence of Animal Charcoal on Water Solution of Kephalin.—To a solution of 1 g. kephalin in 100 cc. of water, 2 g. of pure animal charcoal were added, the mixture shaken, and then subjected to the vacuum filter. A little fluid passed, which became at last quite clear. A portion of the last clearest, collected by itself and tested, was found to be almost pure water; for hydrochloric acid, platinic chloride, barita hydrate, and lead acetate, produced the very feeblest precipitates only, while the original solution was made solid by the same precipitants. The animal charcoal therefore retained the kephalin, and when isolated and extracted with alcohol yielded it up to that solvent. This experience, as well as that made with albumen, shows that the watery solution of kephalin cannot be clarified by these agents, if indeed they do not show also that the condition of kephalin is one of suspension and not of true solution. That, however, charcoal has a special attraction for kephalin, such as it also exhibits towards other ammonium bases and alkaloids of undoubted solubility in water, is shown by the following experiment.

Bearing of Kephalin in Ether with Charcoal.—A concentrated solution of kephalin in ether was treated with much animal char-

coal, in order to be decolorised. The object was but partially obtained. The charcoal, after filtration and washing, was found to retain much kephalin, which was extracted by boiling absolute alcohol, and from this deposited on cooling in a *perfectly white state.* The solution deposited more on spontaneous evaporation. Both were tested and identified as kephalin.

Kephalin therefore can be removed from watery solution by charcoal and curdling albumen, and again extracted from these substances by hot or cold alcohol in large quantity, and obtained from these solutions in a perfectly white state.

Ultimate Analysis of Kephalin.—A specimen of highly purified kephalin was passed through the water-filtration and hydrochloric acid process; it amounted to four litres of one per cent. solution, and after resolution in ether and precipitation by alcohol, left about 30 g. dry matter. It was thoroughly dried in vacuo over sulphuric acid, being frequently triturated, and ultimately reduced to a fine powder. Carbon and hydrogen were determined by combustion with lead chromate and copper turnings. Nitrogen was determined by volume, the bichromate and carbonate mixture being used for production of carbonic acid gas. Phosphorus was determined by evaporating the substance with solution of pure soda, made from metallic sodium, mixed with carbonate and nitre, to dryness, slowly deflagrating, etc., and determining phosphoric acid by magnesia method.

Thus were obtained the following percentages and atoms:

	Found.	÷ by At. Wgt.	÷ by N=1	Theory. Atoms.		Theory. Per cents.
C	60·00	5·00	41·7	42 C	504	60·28
H	9·39	9·39	78·3	79 H	79	9·44
N	1·68	·12	1·0	N	14	1·67
P	4·27	·13	1·0	P	31	3·70
O	24·66	1·54	12·9	13 O	208	24·88
	100·00					

leading to formula $C_{42}H_{79}NPO_{13} = C_{42}H_{69}NPO_8 + 5H_2O$.

We shall see hereafter that this formula is supported by the results of the analysis of a number of other preparations, being partial or complete compounds of kephalin, of which the organic matter always has the composition of the free substance; the results of a series of chemolytic experiments lead to the latter formula. But the assumption of the presence of five molecles of

water of hydration brings the constituent hydrogen to so low a figure that it cannot be explained out of the sum of the chemolytic products. On the other hand, the assumption of the presence of five atoms of loosely attached oxygen meets also with theoretical objections. The difficulty here touched requires evidently experimental elucidation.

Solubilities of Kephalin.—In water kephalin swells and forms an emulsion, ultimately an imperfect turbid solution. Its affinity for water is very great, and the last quantity of water is expelled from it in the vacuum only with great difficulty and after a long time. From a watery solution or mixture it cannot be extracted by ether, as the liquids form an emulsion which persists even after a portion of the ether has separated from the water. This emulsion is very thick, like paste, white like milky water, and practically unmanageable. When a drop or a few drops of a concentrated ether solution are allowed to fall into a test-tube full of water, the mixture is at once transformed into a white jelly, which is so firm that the tube can be turned upside down without anything flowing out of it. Solutions of kephalin in water on standing do not decompose or become mouldy, even in the course of some weeks.

Cold absolute alcohol dissolves a little kephalin, more on boiling, and deposits a part on cooling in white flocks. One hundred parts absolute alcohol at 17° C. dissolve seven parts kephalin; at boiling heat of the alcohol nine parts, of which two parts are deposited on cooling. When an excess of kephalin is boiled with an insufficient amount of alcohol, the part which remains insoluble does not seem to undergo any change, for it retains its solubility in ether and precipitability by alcohol and other reactions.

In ether kephalin, when not too much hydrated, is highly soluble; when dry it is soluble in anhydrous ether in almost any proportions; it does not crystallise from this solution, and cannot be made to deposit as from a mother-liquor. It is precipitated from the ether solution by an equal or greater volume of alcohol in white clouds, which combine to clots, and ultimately forms a firm substance, which becomes at first plastic, and then dries in vacuo to a hard brittle mass. The ether solution becomes quickly red in transmitted light, and fluoresces with a fine green colour. No other phosphorised or other brain ingredient shows this peculiarity except kephaloidin.

A specimen, thrice precipitated from ether by alcohol, and

when last in ether, exposed during twenty-four hours in ice to a temperature of 0°, proved soluble in cold benzol, and very soluble in hot. It formed a yellow solution. A sample in a test-tube exposed to frost gave no deposit. The addition of alcohol to the benzol solution produced a slight precipitate, insoluble in excess of alcohol, but soluble in excess of benzol, and soluble on heating. Benzol can therefore not be used, like ether, for the purification of kephalin.

Reactions of the Aqueous Solution of Kephalin.—A 1 per cent. solution, filtered by air-pressure through three-fold Swedish filter-paper, was used.

1. Hydrochloric acid gives a bulky curdy precipitate, slightly yellow, and after isolation soluble in ether, *not* precipitated by alcohol from its ethereal solution. (In the filtrate from this hydrochloric acid precipitate platinic chloride produces the merest opacity.) But from the ethereal solution of the HCl precipitate alcoholic $PtCl_4$ throws down a precipitate, which is soluble in ether, and reprecipitated by alcohol.
2. Sulphuric acid produces a precipitate like that produced by HCl.
3. Nitric acid the same as the previous acids.
4. Barita water produces a bulky curdy precipitate.
5. Lime-water produces a similar precipitate, but it does not separate like the BaH_2O_2 precipitate.
6. Cadmic chloride induces a curdy precipitate which readily coalesces.
7. Zinc chloride behaves similar to $CdCl_2$.
8. Mercuric nitrate produces a dense precipitate, which is insoluble in nitric acid, but coloured slightly yellow thereby, heat being evolved. The precipitate is sometimes rose-red, and in adhesive flakes. Washed and allowed to stand with water it becomes again white, ropy, and adhesive, and soft, and on being shaken easily dissolves in water in the manner of the original kephalin. The mercuric nitrate seems therefore to be separated by water from kephalin in the same manner as other salts and acids are.
9. Barium chloride produces a good dense flaky precipitate. Immediately after isolation it is insoluble in water, insoluble in alcohol, but easily soluble in ether, and apparently reprecipitated by alcohol. This reprecipitated matter contains barium, but gives it up again to water.
10. Calcium chloride acts like $BaCl_2$.
11. Platinic chloride produces a bulky precipitate.

12. Ammonia makes solution a little turbid, but causes no precipitate.
13. Platinic chloride mixed with HCl produces a very well-defined precipitate.
14. Magnesium chloride causes a very precise immediate precipitate.
15. Ferric chloride produces a yellowish turbidity and imperfect precipitate.
16. Uranic nitrate produces a white turbidity and imperfect precipitate.
17. Watery bromine produces a bulky, nearly white precipitate, soluble in caustic potash; acetic acid added to this again liberates the precipitate. Chloroform added to this mixture produces a chloroform solution of Br at the bottom, containing the excess of reagent, and an impracticable white emulsion on the top.
18. Cupric nitrate
19. Cupric chloride
20. Cupric sulphate
21. Cupric acetate

} All produce perfect precipitates of a greenish-white colour.

22. Mercuric chloride makes the solution very turbid, but produces no precipitate.
23. Mercuric acetate causes an immediate complete precipitate.
24. Silver nitrate, immediate complete precipitate, darkening a little when exposed to sunlight.
25. Gold terchloride, and a drop of hydrochloric acid, cause an immediate precipitate, which blackens over night.
26. Antimonic chloride produces a very bulky precise white precipitate.
27. Stannous chloride, a white flaky complete precipitate.
28. Stannic chloride, a precipitate and turbid solution.
29. Tannin in water, no particular reaction.
30. Picric acid, a turbidity, but no manageable precipitate.
31. Arsenious acid, a precipitate and turbidity.
32. Arsenic acid, a very complete immediate precipitate.
33. Phosphoric acid, a very complete immediate precipitate.
34. Basic lead acetate, a precipitate and very turbid solution; no perfect separation.

In none of the foregoing reactions was any artificial heat employed, but they were all made at the ordinary temperature.

It was found that most of these precipitates could not be washed with water without losing either acid or base, or salt, with which they were combined. But most of them remained insoluble in water until the point of purity was reached, when the kephalin either dissolved in the pure water, or clogged the filtering-paper.

Compounds of Kephalin.

Kephalin Cadmium Chloride.—Two litres of a filtered 1 per cent. solution were precipitated by dilute HCl. The mother-liquor was drawn off, and the precipitate washed by agitation with water. The washing-water was again drawn off, and watery solution of cadmium chloride added, which caused great condensation of the precipitate. The liquor was again drawn off, and the precipitate shaken violently with a great quantity of alcohol containing alcoholic cadmium chloride. Thus the precipitate was condensed to a viscous mass, from which the mother-liquor was drawn off, and the alcohol entirely removed by careful manipulation. After draining, the precipitate was dissolved in ether, and to the ethereal solution was added alcoholic cadmium chloride cautiously, till a slight permanent precipitate was perceived. This was removed by filtration, and the brilliant fluorescent filtrate precipitated by absolute alcohol. The viscous mass was again drained from all alcohol by pressure with a glass rod. Redissolved in ether, it formed a perfectly clear solution, which was reprecipitated by absolute alcohol, when the compound came down in an almost pulverulent state. It was thrown on a filter, washed with absolute alcohol, then removed on a glass dish and placed under a dryer, then in a vacuum over sulphuric acid, and frequently removed to be powdered in a mortar, and ultimately finely pulverised. It was then subjected to elementary analysis. Carbon and hydrogen were determined by combustion with $PbCrO_4$, and copper turnings. Nitrogen was determined as gas. Chlorine, cadmium, and phosphorus were determined by fusion with caustic soda, nitre, and carbonate, solution of salts in acid, precipitation of cadmium by hydrothion, and conversion into carbonate. Chlorine and phosphorus were determined in filtrate from cadmic sulphide by the usual methods. The cadmium and chlorine were in the relation of $Cd:Cl_2$, inasmuch as 4·15 parts chlorine require 6·54 parts cadmium.

Summary of per cent. found:

C	53·603	
H	8·519	
N	1·37	89·38
P	3·54	
O	22·35	
Cd	6·47	10·62
Cl	4·15	
	100·00	100·00

Calculation shows that the kephalin is not completely saturated with $CdCl_2$; that about four parts out of nine are uncombined. For the formula, derived from the organic matter with P as 1, *i.e.*, $C_{42}H_{79}NPO_{13},CdCl_2$ yields the equation—

$$\underbrace{C_{42}H_{79}NPO_{13},CdCl_2}_{1019} : \underbrace{CdCl_2}_{183} = 100 : 17{\cdot}95,$$

whereas only 10·62 per cent. $CdCl_2$ were found.

Deducting $CdCl_2$, and calculating per cents. of elements in organic body, we get—

		÷ by At. Wgts.	÷ by P as 1.	At. Wgts.
C	59·97	4·99	41·6	504
H	9·53	9·53	79·4	79
N	1·53	·11	1·0	14
P	3·96	·12	1·0	31
O	25·00	1·56	13·0	208
				836

leading to formula $C_{42}H_{79}NPO_{13}$, with an atomic weight of 836, thus fully sustaining the composition of the free substance.

As 10 parts of $CdCl_2$, supposed to be combined with a molecle of kephalin, correspond to 59 parts of compound, about 40 per cent. of the above substance may have been free kephalin.

The very weak chemical affinities of kephalin are here exhibited in a striking manner; $CdCl_2$ was brought into contact with it at various periods, and yet could not be retained in combination. This is due to the circumstance that $CdCl_2$ cannot be dissolved in absolute, but only in somewhat watery alcohol; and even little water decomposes the compound, and carries the $CdCl_2$ away, as will be shown by special experiment hereafter.

Kephalin with Hydrochloric Acid and Platinic Chloride.—About three litres of 1 per cent. solution, which by the deposition of the insoluble part and filtration had lost much of the 1 per cent. originally dissolved, were treated with a mixture of HCl and $PtCl_4$ in slight excess. A bulky yellowish-white curdy precipitate ensued, and rose to the surface; it was allowed to contract, and the yellow mother-liquor drawn from beneath it by a siphon. Absolute alcohol, equal in bulk to the precipitate, was now poured upon it, and the mixture violently shaken. This caused a further contraction of the precipitate, indicated by lesser bulk, increase of the yellow colour, and production of adhesiveness. The alcoholic

liquid was again siphoned off, and another quantity of absolute alcohol was now poured on the precipitate, and the mixture violently shaken. The precipitate thereby became deep yellow, adhering to the glass, and so contracted that the liquor could be poured off quite clear. The precipitate was now treated with a minimum of ether, in which it proved quickly and entirely soluble. On filtration, nothing whatever remained on the filter. An equal volume of absolute alcohol was added to the ether solution, whereby almost the whole of the salt was precipitated. The latter was freed from mother-liquor by careful manipulation with a glass rod, redissolved in absolute ether, which gave a brilliant solution requiring no filtration, and this was reprecipitated by absolute alcohol in equal volume, added in a thin stream while the liquid was being stirred. The mother-liquor was poured off; the precipitate, which immediately became brittle and hard on contact with absolute alcohol, was drained from alcohol and placed over sulphuric acid in the vacuum.

Another amount of the same body was made from four litres of a 1 per cent. solution in the same way, with this difference—that whereas in the first case the precipitate was washed with alcohol directly, in *this* case two washings with water were carried out before the application of alcohol. This was done in order to test the effect of water upon the compound desired to be produced. It was found that the dry body treated with ether and alcohol like the first preparation contained only a trace of platinum and a vestige of chlorine, both elements being too small in quantity for accurate determination.

Both preparations were therefore united, and it was sought to combine them with platinic chloride under circumstances where the influence of water was as much as possible excluded. (It must be remembered that solid platinic chloride contains six molecles of hydration water, which it necessarily carries into all its solutions.) They were dissolved in ether, and an ethereal solution of $PtCl_4$ was added, then precipitated with absolute alcohol; again dissolved in ether, and treatment with $PtCl_4$ repeated. Ultimately the precipitate was dissolved in pure ether, reprecipitated by pure alcohol, and dried in vacuo.

Analysis gave 3·592 per cent. Pt, and 3·265 per cent. Cl. If the platinum had been accompanied by only as much chlorine as corresponds to tetrachloride, 2·58 per cent. Cl should have been

found. By calculation we find that there is one-fifth of Cl more than corresponds to this proportion, viz., 1 Pt : 5 Cl = 3·592 Pt : 3·22 Cl.

A compound of the presumable formula :

$2(C_{42}H_{79}NPO_{13}) + 2ClH + PtCl_4$ with an atomic weight of 2083 requires 9·5 per cent. Pt and 10·2 Cl. Consequently, the platinic chloride compound comprises about one-third of the kephalin, of which two-thirds are uncombined.

It is thus seen that although kephalin is most completely precipitated by $PtCl_4$ from its watery solution, yet by the process of solution and precipitation with solvents in which $PtCl_4$ is soluble most of the $PtCl_4$ is extracted from the combination and lost in the mother-liquors, just as it is almost entirely extracted from the same precipitate by water. In short, the acids, bases, and metallic salts which easily combine with kephalin when they are present in excess, are rapidly separated from it by solvents in which they themselves are readily soluble.

Chemically speaking, these results are disappointingly negative, inasmuch as they refuse to furnish the ordinary means for determining the atomic weight and for finding guarantees of purity of preparations. But physiologically these features are of the greatest interest, inasmuch as they show us a marvellous diversity of power of reaction of kephalin, by its entering into and out of combination according to external circumstances. When the combinants are offered in a concentrated state they are retained; when the liquids which carry the combinants (blood, serum, cerebrospinal fluid) become again diluted, the combined matters must again pass into solution and travel further. Thus every change of chemical composition of the juices of the body must necessarily and powerfully affect the condition of the brain and nerves, and of all tissues and cells containing their specific ingredients.

b. Amidokephalin.

The details of the observation regarding this principle, and of its transformation into lead salt, will be given lower down, in the section of the dinitrogenised monophosphatides.

c. Oxikephalin with Cadmium Chloride, $C_{42}H_{79}NPO_{14},CdCl_2$.

When the white matter (Ox) has been extracted with ether and the kephalin removed from the ether solution by precipitation with

absolute alcohol, there remains a bulky solution containing all lecithin, much sphingomyelin, and some kephalin, together with the cholesterin previously contained in the white matter. When to this solution $CdCl_2$ is added, a voluminous precipitate ensues, which, after washing, yields to ether a quantity of coloured salt. This after concentration is precipitated by alcohol, and purified by repetition of this treatment. The composition of this precipitate is shown in the following summary of analyses :

Per cents. found.			÷ by At. Wts.	÷ Cd as 1.	In 100 of organic matter.
C	48·12	81·95	4·01	42·21	58·71
H	7·55		7·55	79·47	9·21
N	1·43		0·102	1·07	1·74
P	3·524		0·113	1·18	4·30
O	21·33		1·33	14·00	26·02
					99·98
Cd	10·65	18·05	0·095	1·00	
Cl	7·40		0·208	2·18	
	100·004	100·00			

leading to formula $C_{42}H_{79}NPO_{14}$, $CdCl_2$.

This compound is noteworthy on account of two features, viz., that it coincides with the composition of the theoretical $CdCl_2$ salt of kephalin, plus one atom of oxigen, and that the $CdCl_2$ is a complete molecle, combined with an apparently complete molecle of organic matter. Such compounds as this and the ones to be described hereafter with 15 atoms of oxigen, make one regret that there are no means of determining oxigen directly in organic chemistry. The oxigen is estimated by the void left by the substances determined, and this gives an opportunity for small impurities to be summed up under the guise of this element. Now the substance here considered had not undergone the process of purification by water, filtration, and acid, and it may therefore have been kephalin to which some slight impurity was attached. On the other hand, there is no proof of the existence of such impurity, and none could be found by testing. It is therefore necessary to consider this substance as a genuine compound of a kephalin containing an atom of O more than the normal kephalin, to which it will be convenient to apply the term *oxikephalin*. In any case the isolation of this body from the mother-liquor of kephalin by the ether process, by means of $CdCl_2$ is of sufficient

importance in itself, no matter how the question of the atom of oxigen may ultimately be decided by further research.

Behaviour of a similar Salt with Water.—A portion of a salt similarly obtained, though not analysed, was digested with water, whereupon it began to swell, and the water after filtration was found to contain large quantities of $CdCl_2$, proved expressly by the hydrothion and silver tests. When the extraction with water had been continued for some time filtration was effected, when during washing the body swelled to such an extent as to clog the filter. There was therefore no guarantee of its purity from $CdCl_2$. The experiment proves that by simple digestion with water much $CdCl_2$ is extracted, but the entire amount can only be removed by a long *process of dialysis* in corrugated filters of vegetable parchment. The compound cannot be freed from cadmium by H_2S, as when so treated in ether solution it only assumes a yellow colour, and the CdS remains dissolved. This peculiar bearing is observed by several phosphorised compounds.

d. Peroxikephalin, $C_{42}H_{79}NPO_{15}$.

A quantity of kephalin, obtained after frosting the ether solution and precipitating it by absolute alcohol, was subjected to elementary analysis, without having undergone the water filtration and HCl process, and gave results of which the following is a summary :

	In 100.	÷ by At. Wts.	÷ by N = 1.
C	57·750	4·8125	42·85
H	8·902	8·9020	79·26
N	1·573	0·1123	1·00
P	3·680	0·1187	1·05
O	28·095	1·7560	15·63

leading to formula $C_{42}H_{79}NPO_{15}$; at. w. = 868.

Transformation of this Body into Lead Salt.—About 10 g. of the analysed substance were dissolved in ether, and to this solution *a warm alcoholic solution of lead acetate* was added. A viscous precipitate was produced, which settled in a mass. The lead acetate was not used in excess, the addition being discontinued while there was still a little matter in solution admitting of precipitation. The mother-liquor was poured off, the mass of the precipitate was stirred and rinsed with a little ether first, and afterwards with absolute alcohol. The precipitate was now digested with a quantity of ether, which dissolved much and left a portion un-

dissolved, which was disregarded. The solution was filtered off and precipitated by absolute alcohol, the precipitate was washed four or five times with absolute alcohol, dried and analysed.

The compound dried at 80° C. was fused with soda, nitre, and carbonate mixture, the lead precipitated by H_2S, and the PbS transformed into $PbSO_4$ by ignition with HNO_3 and H_2SO_4.

Summary:

	Per cent. found	$\underbrace{C_{42}H_{75}Pb_2NPO_{15}}$ requires
C	38·337	39·436
H	5·760	5·868
N	0·9755	1·095
P	2·717	2·425
O	20·312	18·782
Pb	31·869	32·394
	100·000	100·000

It will be seen by a comparison of the oxigen quantities in the free body, with those of the salt, that there is no reason for assuming lead to be present as oxide; on the contrary, the H being less in the organic part of the lead salt than in the free body justifies the assumption of a substitution of H_4 by Pb_2.

Comparison of the Organic Matter in the Lead Salt, with the Composition of the Free Body and of the Organic Matter in a Salt of Kephaloidin with $CdCl_2$.

	Per cent. found in original free peroxikephalin.	Per cent. found in $CdCl_2$ salt of kephaloidin.	Per cent. found in lead salt of peroxikephalin.
C	57·75	57·91	56·31
H	8·90	8·82	8·30
N	1·57	1·67	1·43
P	3·68	3·71	3·98
O	28·09	27·78	29·81

The phosphorus, as in nearly all analyses, is found somewhat too high, rising in the lead salt to 5 as compared with N as 4. But on the whole the change by the removal of some insoluble salt is not so great as to negative the assumption that the free body and body contained in the lead salt have essentially the same composition, more particularly *the proportion of oxigen has not been decreased by the combination.*

e. Kephaloidin.

Definition.—The substance thus designated is much like kephalin as above described and may be identical *in composition* with it,

but presents some slight differences, which necessitate a preliminary distinction. It is obtained from buttery matter; kephalin from white matter. It is more fluid than kephalin when first precipitated, and never dries to the same hard brittle substance as kephalin, but presents a fused appearance. It presents the same irregularities in its combinations as kephalin; *it forms oxikephaloidin*, which, like oxikephalin, combines with a molecle of $CdCl_2$, forming a pretty concise salt.

Solubility in Water and Filtration.—Five g. of dry, hard, but plastic kephaloidin were dissolved in 500 cc. of cold water with trituration. The substance became mucous at first, and on agitation in a long cylinder was disintegrated, and a turbid emulsion-like solution resulted. This was passed through the pressure-filter, and passed easily ten layers of English filtering-paper. Next ten layers of Swedish paper were employed, when an entire atmosphere of pressure allowed the liquid to pass, but slowly.

Bearing in Dialysing Apparatus.—First Experiment.—The foregoing solution, after filtration and subsequent treatment with animal charcoal, which did not make it clear, was distributed over two dialysers of parchment-paper. After twenty-four hours the dialysate gave but slight evidence of containing kephaloidin, by giving a mere vestige of precipitate with lead salt, while the original solution gave a very copious precipitate. Only a very minute portion of matter, therefore, had passed the diaphragm.

Second Experiment.—A solution and emulsion of about 20 g. of kephaloidin in 500 cc. of water was, without having been filtered, subjected to dialysis. After twenty-four hours only a very small quantity of kephaloidin had passed, as shown by the lead precipitate. The original solution on the dialyser gave a copious thick precipitate with the same lead salt. It was thus shown that a 1 per cent. solution can hardly be dialysed, while a 4 per cent. solution dialyses a little, but not enough for practical purposes. Kephalin and kephaloidin act in watery solution like colloids, and remain on the dialyser, while allowing the crystalloids mixed or combined with them to pass in the pure water. They act as dialysers themselves when placed in pure water, and yield up the soluble salts or bases or acids with which they are combined. Dialysis by vegetable parchment is effective in completing this process.

Bearing of the Ether Solution with Water.—The kephaloidin was

twice precipitated by alcohol from ether solution, and then redissolved in ether. A few drops falling in water are precipitated, and on shaking a turbid emulsion is formed. Boiling transforms this into a turbid mucous mass with thick and viscid flakes. Hydrochloric acid added to this emulsion causes white curdling, and the white flakes can with difficulty be filtered off. The filtrate is white and turbid.

Water, hydrochloric acid, and ether in certain proportions produce a thick white jelly; a little more ether separates oily ether solution, which gives no precipitate with alcohol, or in this mixture with $CdCl_2$, and contains therefore HCl.

Ether solution mixed with little water becomes a *solid white mass;* more water produces curds, ultimately emulsion and solution.

Kephaloidin is easily soluble in benzol; treated with HCl gas, this solution changes colour, but gives no precipitate; alcohol added to this gives a little precipitate, soluble in excess; ether gives no precipitate in the benzol HCl solution. A solution of kephaloidin in anhydrous ether is not precipitated by benzol.

Reactions of the Watery Solution of Kephaloidin:

Hydrochloric acid produces a dense slightly yellow precipitate, which after isolation is soluble in ether and not precipitated by alcohol. In the ethereal solution of HCl precipitate, alcoholic $PtCl_4$ produces a precipitate soluble in ether and reprecipitated by alcohol.

Sulphuric acid produces a precipitate like that by HCl.

Nitric acid, same as sulphuric.

Barita water, Lime water, Cadmium chloride, Zinc chloride: All produce good precipitates which coalesce well on standing or agitation.

Mercuric nitrate gives a good curdled precipitate insoluble in HNO_3, but made slightly yellow thereby.

Barium chloride also produces a good flaky precipitate. This body after isolation is insoluble in water and absolute alcohol, but readily soluble in ether.

Platinum chloride gives a complete precipitate.

Lead acetate gives a good precipitate.

Mercuric acetate gives a very voluminous precipitate.

Cupric acetate, a whitish flocculent precipitate.

Kephaloidin Lead.—A specimen of kephaloidin which had been frozen in the ethereal solution, and precipitated by alcohol, was

once more dissolved in ether, and placed in a freezing mixture for twenty-four hours. No deposit occurred. This ether solution also gave the reactions above described, and with silver nitrate its watery solution gave a copious white precipitate. The ether solution was poured slowly in a thin stream into absolute alcohol, when the kephaloidin was precipitated as a viscid mass. The whole of this was dissolved in water, and lead acetate added; a copious precipitate ensued, which was filtered and washed, extracted with warm dilute alcohol, ultimately with warm absolute alcohol; it then *dissolved in ether without residue,* was precipitated by absolute alcohol, became pulverulent, was dried in vacuo, and analysed.

Summary.			In 100 organic matter.	Theory of $C_{42}H_{79}NPO_{13}$.
C	50·983	Total organic 83·740	60·88	60·28
H	7·721		9·22	9·44
N	1·017		1·21	1·67
P	3·666		4·37	3·70
O	20·353		24·32	24·885
Pb	16·260	16·260	100·00	100·000
	100·000	100·000		

It is at once evident that the Pb stands in no simple proportion to any other element. Kephalin lead if dibasic would require 19 per cent., if monobasic 11 per cent., of Pb. The molecle of the kephaloidinate contains thus rather more than half a molecle of Pb, and is consequently a mixture of lead salt with free body. There is also an irrationality perceptible on the P, which is too high, and the N, which is too low. But on the whole the constitution, properties, and products coincide with those shown by kephalin of the compared formula.

f. Oxikephaloidin with Cadmic Chloride, $2(C_{42}H_{75}NPO_{14}) + CdCl_2$.

In this case a quantity of kephaloidin obtained by the ether process, not purified by water, filtration, and HCl, etc., was transformed into $CdCl_2$ salt, and precipitated by alcohol; redissolved in ether, and the solution repeatedly frozen, and freed from some deposit, then precipitated by absolute alcohol. It could be dried at 80° C. without change.

Summary of analyses per cent.:

C	52·796	Total organic 91·160
H	8·142	
N	1·500	
P	3·690	
O	25·032	
Cd	5·410	8·840
Cl	3·430	
	100·000	100·000

leading to formula $C_{42}H_{75}NPO_{14}$ for organic matter. If one atom of $CdCl_2$ were combined with two atoms of organic body, then 9·8 per cent. $CdCl_2$ should have been present.

Comparison of the Composition of the Organic Matter with

Per cents. of

	Organic matter of oxikephalin with $CdCl_2$.	Organic matter in oxikephaloidin with $CdCl_2$.	Free peroxikephalin.	Organic matter in lead salt of peroxikephalin.
C	58·71	57·91	57·750	56·31
H	9·21	8·82	8·902	8·30
N	1·74	1·64	1·573	1·43
P	4·30	4·04	3·680	3·98
O	26·02	27·45	28·095	29·81

It will thus be perceived that the oxikephaloidin is intermediate between oxikephalin and peroxikephalin in composition in all items except alone hydrogen. This peculiar anomaly, if such it be, must be reserved for future deliberation. The empirical formula expressing the composition of this salt is $2(C_{42}H_{75}NPO_{14}) + CdCl_2$.

The ethereal solution of this salt was not precipitated by hydrothion gas passed through it.

g. Decompositions of Kephalin.

When pure kephalin in the perfectly dry state is heated in a water-oven to between 90° and 100° it fuses to a dark red transparent viscid oil. It becomes solid again on cooling, but retains a viscosity, so as to adhere to the fingers, which it did not before it was heated. Treated with water, the heated and cooled kephalin swells again and gradually dissolves, but is darker

coloured, so that some slight degree of decomposition or of oxidation seems to have taken place.

When heated to higher temperatures it gives out much heavy strongly smelling inflammable vapour, which partially burns, with formation of much soot. Ultimately a bulky charcoal is left, which cannot by ordinary heating in platinum vessels be entirely destroyed, as it is soaked with phosphoric acid. It cannot be entirely burned, even after the principal quantity of phosphoric acid has been extracted with water. Complete combustion is effected only in the presence of nitre.

With concentrated sulphuric acid dry kephalin in fine particles immediately assumes a dark red-brown colour, which gradually becomes nearly black. When sugar and sulphuric acid are allowed to act upon kephalin, a reaction similar to the one given by bile-acids is gradually engendered. But the process essentially requires time, during which the mixture passes through a stage of dark-brown colour, until a deep purple is at last attained. But this colour is never so pure or beautiful as that obtained with cerebrosides, myelin, or the bile-acids. It is therefore probable that a decomposition of the kephalin has to be effected before the reaction is attained.

h. Chemolyses of Kephalins.

a. Limited Chemolysis by Caustic Soda.—A watery solution containing 30 g. of pure kephalin, filtered, etc., was precipitated by HCl. The pulpy deposit was put into a flask, and a solution of 5 g. crystallised soda hydrate added. The precipitate disappeared on agitation, forming first a gelatinous, later a fluid solution. The solution was now placed in a bath, and heated gently for nine hours. During the process it was observed that skins formed on the surface similar to membranes on milk while being heated. On cooling, the liquid formed a gelatinous cake, consisting of viscous curds set in a thinner fluid. Next day the mixture was boiled during nine hours on a sand-bath. Bumping was mitigated by dropping a spiral of platinum into the fluid. Great frothing ensued, which was opposed by a funnel fixed upon the top of the flask by a cork, and supported by a stand. After this boiling the liquid was still turbid, became gelatinous, viscid, and set on cooling, and covered by a membrane. On commotion it showed

the wavy glistening appearance of soaps, and when at repose in a beaker formed folds as from a tubular membrane sinking.

Reactions of the Soapy Solution.—It was insoluble in water, and not further precipitated thereby. It was curdled by cold alcohol, dissolved by hot, leaving, however, some particles undissolved, which proved soluble in ether. It was not precipitated by concentrated sodium chloride solution—*i.e.*, not salted out.

Decomposition of the Soaps by Hydrochloric Acid.—The soap was now treated with hydrochloric acid, until a strongly acid reaction was attained, when a whitish-yellow precipitate fell, and the fluid lost its viscosity. The precipitate was washed with water, and the filtrate was evaporated on water-bath.

The Precipitate of Fatty Acids on the Filter was washed, and became very adhesive, yet fatty; boiled in water it did not fuse, but agglutinised a little; nothing like oil or fused fatty acid appeared. Warmed on paper it did not fuse like fat, and gave only a very slight grease-stain; most of it remained slightly glistening on the surface, even when heated until brown. The matter had a fine, smooth, greasy touch between the fingers, and seemed like a fatty acid in a hydrated, swelled state. It dissolved easily in cold absolute alcohol, leaving a quantity of adhesive dark matter undissolved. The latter will not be considered any further in this place.

The Acids soluble in Alcohol—Kephalophosphoric, Kephalic, and a Third Acid.—The solution showed a feeble green fluorescence; it was filtered from a slight secondary deposit of insoluble acid. Alcoholic acetate of lead was now added, which produced a bulky nearly white precipitate. This was washed with absolute alcohol, drained on paper, dried, and treated with ether. It was entirely insoluble in ether, and constitutes

Kephalophosphate of Lead, so named from being the lead salt of a phosphorised acid obtained from kephalin, more complicated than glycerophosphoric acid. The total quantity of salt obtained weighed 6·2 g. Powdered and dried at 95° C., it baked together a little on surface, but when stirred remained pulverulent.

A combustion for nitrogen gave only a trace of permanent gas, so that the substance is proved to be free from nitrogen.

Phosphorus calculated from the Pb pyrophosphate found = 3·572 per cent. P.

Found in 100.		÷ by At. Wgts.	÷ by P = 1
C	48·262	4·022	35·3
H	7·990	7·990	70·1
Pb	23·840	0·1151	1·01
P	3·572	0·1139	1·00
O	16·336	1·021	8·96
	100·000		

leading to formula $C_{35}H_{70}PbPO_9$.

Deducting the lead, and calculating the 76·16 per cent. of organic matter as 100, we get—

		÷ by At. Wgts.	÷ by P as 1.	At. Wgts.
C	63·369	5·2807	34·9	420
H	10·491	10·4910	69·3 + 2	72
P	4·691	0·1512	1·0	31
O	21·449	1·3405	8·8	144
	100·000			667

The atomic weight derived from the Pb pyrophosphate is 663, which closely approximates the quantity found directly.

The conclusion drawn from this experience was that chemolysis, with an amount of alkali insufficient to satisfy at least three molecles of acid, developed from one molecle of kephalin, must stop short of complete decomposition, and produce intermediate products, of which the principal one is kephalophosphoric acid. It is probably an acid containing three acid radicles and an alcohol radicle—

$$\left.\begin{matrix}\text{Kephalyl}\\ \text{Stearyl}\\ \text{Glyceryl}\end{matrix}\right\} \text{Phosphoryl} = \text{Kephalophosphoric Acid}$$

but no nitrogenised nucleus, and by further chemolysis may split up into the acids obtained by its side.

Kephalic Acid and Third Fatty Acid, and their Barium Salts.—The alcoholic solution, from which kephalophosphate had been precipitated by lead acetate, was distilled to about 300 cc. This liquor, on cooling and standing, deposited an oil, which was isolated, and found to float on water; but on boiling, it became *viscous and solid in the hot.* Oil and liquid were treated with ammonia to strong alkalinity, whereby a white emulsive solution was produced. To this, water and watery acetate of lead were added, until, and as long as, a precipitate was produced. This was white, adhesive and bulky. It was filtered, washed, and

dried, powdered, dissolved in ether, filtered, decomposed with hydrochloric acid and water; the ether solution was washed, filtered, and when clear distilled to small bulk. The red solution was treated with watery ammonia, filtered, barium chloride added, when a bulky precipitate was obtained. This was washed for a long time, until washings were nearly free from barita; the precipitate was dried in air, and treated with ether; a coloured salt dissolved and a white salt remained undissolved.

Coloured salt, soluble in ether = Kephalate of barium.

The kephalate of barium dissolves rapidly and abundantly in ether, and is precipitated from this solution by absolute alcohol. It is always of a dark colour, which intensifies in the ether, probably by oxidation. The salt is insoluble, or but little soluble, in boiling alcohol, and is not deposited from this solution as oleate of barium is. The acid cannot be decolorised by animal charcoal, either when in the state of sodium salt, or when in the free state dissolved in boiling alcohol, and all operations seem only to assist in furthering its oxidation. It seems that its radicle, while in the kephalin, is the principal cause of the assumption of colour and fluorescence by that principle.

White salt, insoluble in ether.

This barium salt appears very much swelled in ether; when dry it is white and pulverulent. Hydrochloric acid and water extract the barium, and washing with water leaves the acid soluble in ether; the ether distilled off leaves the acid as a coloured soft mass, which fuses at 26°, congeals at 25°, and crystallises in rosettes like margaric acid. It dissolves easily in absolute alcohol, forming colourless fluid; a few drops of water added to alcohol cause acid to separate as an oil on top of spirit; on standing, colourless rosettes of crystals form in this oil, and may be separated. The spirit deposits white clouds of the acid.

This acid differs from oleic by its barium salt being entirely insoluble in boiling alcohol. It differs from stearic by its lead salt being soluble in ether. It is not fluid at ordinary temperatures, but semi-solid, and fuses only at 26°, and from concentrated spirit it is entirely separated and crystallises, while oleic acid remains dissolved in such spirit, and does not crystallise at such temperatures.

In the manipulation of these acids in the presence of absolute

alcohol the formation of ethylic ethers, which easily ensues, has to be carefully avoided.

Glycerophosphoric Acid.—The acid filtrate containing that phosphorus which was not combined with the kephalophosphoric acid in the form of glycerophosphoric acid, some nitrogen in an unknown form, the sodium chloride, together with some free hydrochloric acid, was evaporated to a low bulk, and its acidity carefully neutralised by caustic soda. Acetate of lead was now added as long as a precipitate was produced, and the white deposit was filtered off. It was boiled, filtered, and washed with hot water, decomposed with H_2S, the filtrate neutralised with calcium carbonate and lime water, filtered and evaporated. When concentrated the solution deposited crystalline *calcium glycerophosphate.*

Neurin and Second Oily Base.—The solution from which glycerophosphoric acid has thus been removed was freed from lead by H_2S, the acetate was decomposed by repeated addition of hydrochloric acid and evaporation to dryness, and the crystalline magma was treated with absolute alcohol to extract the hydrochlorate of the expected nitrogenised body. To this alcoholic solution $PtCl_4$ was added, when a slight yellow precipitate ensued, which was separated by filtration, washed with alcohol, and dried. It was neurin-hydrochlorate-platinic chloride. It could only represent a small portion of the nitrogen contained in the chemolysed kephalin; as the volatilised vapours had not been collected, some volatile alkali may have escaped unnoticed. The *alcoholic mother-liquor* of this platinum precipitate was freed from platinum by H_2S. Evaporated, it left a syrupy residue ; this was again treated with little alcohol and $PtCl_4$, and a slight deposit removed. The liquor on addition of much ether deposited an *oily body*, soluble in absolute alcohol, reprecipitated by ether. Distilled with dilute H_2SO_4 and MnO_2, it gave a distillate which smelled of *acetic acid*, and after neutralisation by soda, was reduced by $AgNO_3$ and by $HgNO_3$, consequently contained *formic acid.* These may be considered as decomposition products of *glycerol*, or of a body *containing its radicle.*

Summary of Results of First Chemolysis of Kephalin.—All first products are soluble, the soaps imperfectly in water. Hydrochloric acid precipitates a mixture of fatty acids.

1. Kephalophosphoric acid, consisting probably of kephalic, a

second fatty acid, and glycerol and phosphoric acid, yet in combination, therefore imperfectly chemolysed.

2. Kephalic acid.
3. A second fatty acid.
4. Glycerophosphoric acid.
5. Ammonia.
6. Glycerin.
7. A base giving oily $PtCl_4$ salt, and being perhaps glyceramin of a new type. Its solubility in water, alcohol, as hydrochlorate and as $PtCl_4$ salt is very great, and its precipitation by ether by no means complete.

β. Complete Chemolysis of Kephalin by Caustic Soda.—40·7 g. of kephalin, purified by the water solution, filtration, and HCl process, as before described, were mixed with 5 g. of crystalline soda hydrate dissolved in two litres of water. The same phenomenon was observed during the boiling as in the first experiment. The boiling was continued during about eighteen hours, of which three hours took place in water-bath, and the other fifteen hours on sand-bath, the time taken to heat it to boiling on the three days during which experiment lasted not included. While on the first and second day the mixture had set into a jelly, it did after the last boiling not become gelatinous again, but remained a somewhat turbid thick fluid. To this HCl was added until the precipitate was curdy and the fluid strongly acid. The precipitate was separated by the filter. It could be washed but imperfectly, as after consolidation on the filter, the precipitate set into a gelatinous tremulous solid mass which allowed no washing water to pass. This mass of acids was placed into a wide-mouthed bottle, shaken with water, and treated with caustic ammonia. It formed a complete solution, which was filtered on hot funnel and left no trace of residue. The solution was opaque, and on agitation showed the silky clouds common to soaps. To this solution acetate of lead was added until the precipitate was curdy, and the liquid distinct and filterable. It was now passed through French filtering-paper, and the precipitate washed with much water, being agitated constantly with a glass rod, so that all parts were well penetrated by water. When the filtrate was free from chlorine and from lead (from excess of acetate), the precipitate was allowed to drain on paper and a cloth and dried. It took many days to dry, being frequently crushed and stirred.

Ether extracted a coloured body, and left a white salt insoluble. This was separated by filtration, and washed by being twice removed from filter, and shaken in a bottle with ether. Filtration was always difficult, owing to the finely divided state of the salt.

The white Pb Salt insoluble in Ether was dried in the vacuum, and after preliminary analysis was further transformed into barium salt by HCl, water, and ether, solution of acid in NH_4HO, and precipitation by $BaCl_2$. The salt was extracted by boiling alcohol, and what remained insoluble was analysed. It was found to be principally *stearate.*

The coloured Pb Salt soluble in Ether was red in transmitted light, and fluoresced green, altogether appeared like a solution of kephalin. It was concentrated by distillation, and the solution precipitated by absolute alcohol; a viscous salt fell down, which became hard in alcohol, was drained and dried in vacuo. After preliminary testing, it was transformed into Ba salt by HCl, water and ether; solution of acid in NH_4HO and precipitation with $BaCl_2$; solution in ether and precipitation by alcohol. It was *kephalate.*

Treatment of the Filtrate containing Glycerophosphoric Acid and Ammonium Base.—It was neutralised with barita water, of which a slight excess was added. A saturated solution of *lead chloride* in water was now added, which produced a flocculent white precipitate of glycerophosphate. The advantages of the lead chloride were that no new acid was introduced into the fluid, from which the ammonium base had yet to be extracted. The fluid became slightly acid, and was corrected by barita water cautiously added. When all was precipitated the *glycerophosphate of lead* was filtered off, washed, and dried. The mother-liquor was concentrated, and then gave another not inconsiderable precipitate with lead chloride solution. The reaction was continued until a filtered sample of liquid remained clear with the chloride, and the second precipitate was isolated and united with the primary one. The previously feebly alkaline liquid was now feebly acid; it was evaporated to a low bulk and then to dryness. It was now dissolved in a minimum of water, and precipitated with absolute alcohol, until this reagent produced no further turbidity, and no deposit on standing twelve hours. The alcoholic extracts were united, concentrated, and precipitated with $PtCl_4$. A yellow precipitate fell, which will be described further on.

The Glycerophosphate of Lead dried to a hard slightly coloured mass. (This hardening of glycerophosphates is also observed upon barium salt, and the condition must be borne in mind when it is subsequently intended to decompose these salts.) It was repeatedly crushed, ultimately powdered, and dried in water-oven. The total weighed 7·4435 g. Now assuming the 40·7 g. kephalin to have contained (at 4·2 per cent. P) 1·7 g. phosphorus, then this corresponds to 19·38 g. glycerophosphate of lead, of which theory is—

Acid.		Pb salt.	Pb pyrophosphate.	
3 C	36	36	—	
9 H	9	7	—	
P	31	31	2 P	62
6 O	96	96	7 O	112
	172	Pb 207	2 Pb	414
		377		588

But as only 7·4435 g. were obtained there is a deficiency of 11·9365 g. Thus much was therefore decomposed further into phosphoric acid and glycerol.

In order to ascertain approximately the purity of the lead glycerophosphate, 0·9110 g. was burned until white, and left 0·7440 g. $Pb_2P_2O_7$, while theory required 0·7104. The white residue was treated with acetic acid and found insoluble in it, which corresponds with pyrophosphate.

The lead salt was now decomposed with hydrothion, the filtrate treated with CO_2 in the cold to expel H_2S, and then with milk of lime to alkalinity, filtered and treated with some CO_2. The filtrate after twelve hours' standing was evaporated near the boiling-point, and filtered hot from the white precipitate of calcium glycerophosphate. This when dry weighed only 0·727 g. Of this 0·3400, after strong ignition with HNO_3, left 0·207 g. residue, equal to 60·8 per cent. $Ca_2P_2O_7$. Pure glycerophosphate should leave 60·5 per cent. of pyrophosphate.

Acid Glycerophosphate of Calcium.—The aqueous filtrate from which the above salt had been removed whilst hot, was mixed with three volumes of alcohol, when a light bulky precipitate ensued. This was isolated by filtration, washed with alcohol and dried. It weighed 0·526 g. Of this 0·2446 g. gave 0·1250 g. pyrophosphate, equal to 51·1 per cent. residue. This corresponds to a

salt which might be obtained from acid glycerophosphate of calcium by combustion.

$C_3H_7CaPO_6 + C_3H_9PO_6 = C_6H_{16}CaP_2O_{12}$ = acid glycerophosphate of calcium.

This on combustion may lose $C_6H_{14}O_5$, and leave $H_2CaP_2O_7$, or half-saturated acid pyrophosphate of calcium, or a body isomeric with it. By this theory 56·54 per cent. residue should be left, which differs so much from 51·1 per cent. found, that we must suppose the salt to have been one of those peculiar alcoholohydrates which salts of glycerophosphoric acid are prone to form, as will be specially proved lower down. There also the actual production of the acid calcic glycerophosphate, here for the first time theoretically assumed, will be proved by further experiments and analyses.

The quantity of glycerophosphoric acid lost in the process of transformation is enormous, and this is invariably observed in all experiments which I have made.

The Platinic Chloride Precipitate.—The yellow precipitate was washed with absolute alcohol. On drying it became horny, crumbled up, was viscous outside and discoloured, and adhered strongly to the paper. Water restored yellow colour and pulverulence. It dissolved in hot water, of which much was required. On cooling, brilliant small crystals were deposited amounting to 0·9428 g. This by analysis proved to be almost pure ammonium salt, containing only a trace of potassium, which raised the residue left on combustion to 45·56 per cent., while 44·36 per cent. are required by pure NH_4 salt. By evaporation over H_2SO_4 two further small crops of ammonium salt were obtained; the rest of the solution dried up to a thick liquid *and did not crystallise.*

The filtrate from the $PtCl_4$ salt was mixed with a large excess of ether until this produced no further turbidity. An *oily matter* settled, which was purified by repeated solution in alcohol and precipitation by ether. It was not analysed, as there were no guarantees of its purity. It was distilled with H_2SO_4 and MnO_2, and yielded an acid distillate, in which, after neutralisation with soda, the presence of *formic acid* was signalised by the usual tests.

This platinum salt, which from the reactions detailed may split up into glycerol and ammonia, reduces the platinum rapidly when

left in contact with ether-alcohol, and becomes black. Altogether this product is one of the most difficult matters to treat, and particularly as it is only obtained in small quantities.

γ. *First Chemolysis of Kephalin with Barita Hydrate.*—The kephalin was prepared by the HCl process, and well washed with water. The quantity used was 27·8 gr. It was placed in a flask connected with a condenser, and a solution containing 80 gr. barium hydrate was added. The mixture was boiled during five hours on a sand-bath, when the precipitate became adhesive and flask cracked. After cooling and filtering the residue was twice boiled with water, during which it became quite soft and semifluid; it was again thrown on a filter and washed to neutrality. The washings were united with the first filtrate.

The barium salts being drained of water, were rubbed in a mortar with ether, and then shaken in a bottle with much ether. The ether *dissolved a coloured salt*, leaving a *white salt undissolved*, from which the solution was separated by filtration.

The insoluble white salt was dried in air; later in vacuo. It was *stearate.*

The soluble coloured salt had the appearance of kephalate, was red, and fluoresced green. The solution was concentrated and precipitated by absolute alcohol. The precipitate was dried in vacuo, and on subsequent analysis was found to be *kephalate.*

The liquid containing the glycerophosphate and ammonium base was treated with carbonic acid until no more precipitate was produced, filtered, and the filtrate concentrated on a water-bath nearly to dryness. During this evaporation it did not deposit any salt, as does the lime salt. The viscous mass was diluted with a little water to fluidity, placed in a bottle, and mixed with absolute alcohol until no further precipitate was produced. The precipitate was filtered off and washed with alcohol.

The precipitate consisted of barium glycerophosphate. It was bulky, white, and granular, probably alcoholate-hydrate. On standing, it contracted, and became horny, transparent, and partly fused. Placed in a glass dish, it fused entirely in a few days, and then dried to a brittle mass.

Summary of Analyses:

C	12·611
H	2·933
Ba	40·950
P	9·266
O	34·240
	100·000

Formula.—$C_3H_7BaPO_6,H_2O$.

The alcoholic solution from which the glycerophosphate had been removed was tested, but the products, being small in quantity, were not analysed. Analyses of this part of the products of the chemolysis of kephalin are given in the following experiment.

δ. *Second Chemolysis of Kephalin by Barita.*—In this experiment, for which a compound of kephalin with cadmic chloride was taken, two sets of barium salts were again obtained, one *soluble*, the other *insoluble in ether*. The salt which was soluble in ether furnished a lead salt, which was likewise soluble in ether (28·056 per cent. Pb), and insoluble in, and precipitated by, ether-alcohol. The salt which was insoluble in ether, when transformed into lead salt, was found to consist of two compounds, one insoluble in ether and in alcohol, the other soluble in ether, but precipitated from it by the addition of alcohol.

The baritic solution was freed from excess of barita by carbonic acid, and the filtrate, after evaporation to a low bulk, was treated with absolute alcohol. A precipitate of barium glycerophosphate was obtained, and identified by quantitative analysis. The alcoholic mother-liquor was neutralised with hydrochloric acid, and precipitated with platinic chloride. The platinum salt so obtained was recrystallised from water, and ultimately obtained in brilliant plates and needles.

Computation of Analyses:

	Percentage.	÷At Wgts.	÷Pt = 1.
C	19·801	1·650	10·1
H	4·726	4·726	29·0
N	4·404	·314	1·9
O	4·701	·293	1·8
Pt	32·219	·163	1·0
Cl	34·149	·961	5 9

$$= (C_5H_{13}NO)_2(HCl)_2PtCl_4.$$

Neurin hydrochlorate platinic chloride.

Besides this salt, there was obtained from the alcoholic mother-liquor from which it had been originally precipitated, a smaller quantity of a second crystallisable platinic salt. After recrystallisation from water it gave the following numbers on analysis :—

Computation of Analyses:

	Percentages.	÷At Wgts.	÷Pt = 1.
C	9·158	·763	4·1
H	3·134	3·134	16·8
N	5·418	·387	2·0
O	6·275	·392	2·1
Pt	36·718	·186	1·0
Cl	39·297	1·107	5·9

$$= (C_2H_7NO)_2(HCl)_2PtCl_4.$$

This salt might be considered as dimethylamin, in which the third atom of hydrogen is replaced by hydroxyl, or as oxethylamin simply.

$$\left.\begin{array}{c} C_2H_5O \\ H \\ H \end{array}\right\} N$$

that is to say, a body formed from neurin, by the loss of three radicles of methyl and one of water.

A third base was also obtained; it was soluble in alcohol as platinic salt, giving the solution a brownish-red colour, and on the addition of much ether was precipitated. It was purified by repeated solution in alcohol and reprecipitation by ether. Several grammes of this body which was precipitated in an oily state, but became solid on drying, were obtained. It was dried at 100° C. and analysed.

Computation of Analyses:

	Percentages.	÷At Wgts.	÷Pt.
C	12·019	1·001	5·0
H	3·070	3 070	15·3
N	5·019	·358	1·79
O	4·255	·265	1·3
Pt	39·466	·20	1·0
Cl	36·171	1·018	5·0

leading to a formula $C_5H_{14}N_2O,HCl,PtCl_4$. This body, so anomalous in its constitution, has probably been derived from neurin molecles by duplication and subsequent decomposition. Such a condensation is observed upon the neurin molecles under the influence of oxidising agents.

ε. *Third Chemolysis of Kephalin by Barita.*—The process was the same as in the former experiment. The platinum salt gave on analysis the following results :

Computation of Analyses :

	Percentages.	÷ At Wgts.	÷ Pt = 1.
C	19·981	1·665	10·3
H	4·719	4·719	29·3
N	4·735	·338	2·0
O	4·443	·278	1·7
Pt	31·832	·161	1·0
Cl	34·290	·966	6·0

$$= (C_5H_{13}NO)_2(HCl)_2PtCl_4.$$

ζ. *Fourth Chemolysis of Kephalin by Barita.*—This experiment was undertaken upon a larger amount of kephalin than the previous ones. The kephalin was first warmed in water, whereby it swelled and became a paste, which was then boiled with two molecles of BaH_2O_2 in the ordinary way.

The same barium salts as those obtained in the earlier experiments were again found, and it was moreover observed that one of the fatty acids contained in them gave the purple reaction, with sugar and sulphuric acid, which resembles Pettenkofer's test for biliary compounds.

From the solution drawn off from the barium salts of the fatty acids the excess of barium was removed by CO_2, and the resulting filtrate concentrated by evaporation, and then treated with alcohol. The glycerophosphate of barium thus obtained was converted into lead salt, and the lead salt into calcium salt ($C_3H_7CaPO_6$).

The alcoholic mother-liquor was freed from alcohol by evaporation, and the resulting solution after addition of nitric acid was precipitated by phospho-molybdic acid, and the product decomposed by hot BaH_2O_2. The yellow filtrate freed from the barium which admitted of removal by CO_2, did yet contain barium in combination. This was proved by combustion of the dried salt and analysis of the residue.

The concentrated solution of the base was freed from Ba by an equivalent amount of very dilute sulphuric acid, then neutralised by hydrochloric acid, and precipitated after concentration by alcoholic $PtCl_4$. The precipitate was recrystallised from water, 10 gr. being obtained in the shape of crystallised plates. The salt was dried and analysed.

Computation of Analyses :

	Percentages.	÷At Wgts.	÷Pt=1.
C	19·47	1·622	9·7
H	4·94	4·94	29·7
N	4·04	·288	1·7
O	4·36	·272	1·6
Pt	32·85	.166	1·0
Cl	34·34	·967	5·8

$$=(C_5H_{13}NO)_2,(HCl)_2PtCl_4.$$

The alcoholic mother-liquor from which this salt had been precipitated gave by precipitation with ether a quantity of the fluid oily salt, the analysis of which has been given in a former para graph, $(C_5H_{14}N_2O)HClPtCl_4$, but it was very small in amount.

A third base was also obtained in small quantity by simple addition of much nitric acid to the original solution, after removal of glycerophosphate, and before precipitation with phosphomolybdic acid, and which gave the various alkaloidal tests.

η. Fatty Acids produced in the Chemolysis of Kephalin from Barium Salt Insoluble in Ether.—The barium salt insoluble in ether was decomposed, after complete extraction with ether and alcohol, by boiling with hydrochloric acid. The liberated acid which solidified upon cooling was dissolved in ether, washed with water so long as it removed colouring-matter, and the ethereal solution was distilled to dryness. The acid which now remained was dissolved in warm ammonia-water, filtered hot and precipitated with acetate of lead. The lead salt after being dried was extracted with much ether, which effected a separation into two salts :

A white lead salt insoluble in ether.
A coloured lead salt soluble in ether.

The Lead Salt Insoluble in Ether. First Preparation.—It was decomposed with tartaric acid in presence of ether and water, and the ethereal solution of acid after washing was treated with animal charcoal, which clarified and partially decolorised it. The acid obtained by distillation of the ether was repeatedly crystallised from watery alcohol. Finally four crystallisations were obtained, dried by fusion for some time at 105° C., and their melting-points determined. No. 1 fused at 68°; 2, at 66°; 3, at 62·5°; 4, at 63·5° C. These preparations were united and recrystallised from dilute alcohol with animal charcoal. They finally gave one large crop of acid melting at 68° C., from

the mother-liquor of which a small further quantity was obtained, melting at 66° C. It was stearic acid, mixed with a small quantity of a lower homologue.

Second Preparation.—Another portion of lead salt was decomposed with tartaric acid and ether, and the ether distilled to a low bulk. On cooling, a large crop of nearly white crystals was obtained. After separation from the highly coloured mother-liquor by filtration and pressing, the acid was twice recrystallised from dilute spirit with animal charcoal. There was thus obtained a perfectly colourless acid, having a fusion-point of 70° C. This was dried for analysis by fusion at 110° C., after which treatment its fusion-point was found still the same. Analysis led to the empirical formula $C_{18}H_{36}O_2$, which is that of stearic acid. This acid has the same melting-point, microscopic appearance, and other physical characteristics as stearic acid. A portion of it dissolved in dilute ammonia gave a salt which dissolved perfectly on heating, almost completely separated in crystals on cooling, and otherwise exactly resembled stearate of ammonium prepared from pure stearic acid.

Third Preparation.—Obtained by chemolysis with hydrochloric acid. The insoluble in ether lead salt, from insoluble in ether barium salt obtained in this chemolysis ('Report,' 1876, p. 118), was examined as to its identity with the analogous salt obtained in the barita chemolysis. It was decomposed with tartaric acid in presence of ether, and the ethereal solution was distilled, and when sufficiently concentrated was allowed to crystallise. The crystals, purified by recrystallization from watery alcohol with the aid of animal charcoal, gave a white product, which fused at 69° C. and was analysed. In all respects it was identical with stearic acid.

Lead Salt Soluble in Ether.—The clear ether solution was distilled to dryness, again dissolved in ether, decanted from matter rendered insoluble, and treated with a concentrated solution of tartaric acid. The ethereal solution of the acid free from lead gave on evaporation a viscid mass which, being dissolved in ammonia and precipitated with barium chloride, gave a viscous barium salt. This yielded to boiling alcohol a small amount of a salt which was analysed. It contained 19·65 per cent. of barium. This body requires much further study.

Barium Salt Soluble in Ether, Kephalate. Product of the first

Chemolysis.—This salt dissolved in ether with a red-brown colour, from which it was impossible to free it by any process whatever. To try whether this colour were due to oxidation caused by contact with the air, some of the free acid was enclosed in a tube with a measured volume of oxigen gas, but no absorption was observed. It is therefore clear that any oxidation must take place in ethereal solution, and in that case must be ascribed to the peroxide of hydrogen formed by the ether. The red-brown colour appears to be proper to the acid and its salts. To ascertain whether the soluble in ether barium salt contained more than one acid, it was decomposed with tartaric acid in presence of ether, and the liberated acid obtained upon distillation to dryness was dissolved in ammonia and precipitated with acetate of lead. The lead salt was dissolved in ether, and fractionally precipitated in three portions by successive additions of alcohol. The fractions were analysed, and gave 38·07, 38·48, and 36·39 per cent. lead.

It is therefore probable that the soluble barita salt mainly consists of one acid only. The following attempt was made to obtain it in a state fit for analysis.

After the barium salt had been exhausted with boiling alcohol it was dissolved in ether, and the intensely coloured solution precipitated by alcohol; the precipitate was redissolved in ether and again precipitated. The mother-liquors removed no colouring-matter. The compound was next decomposed by tartaric acid, the free acid dissolved in ether; the solution was distilled to dryness, and the residue extracted with absolute alcohol. The solution thus obtained was treated with ammonia in excess, and the filtered clear soap solution precipitated with acetate of barium. The precipitated salt was analysed.

	Percentages.	÷At. Wgts.	÷Ba = 1.
C	59·556	4·963	37·3
H	8·488	8·488	63·8
Ba	18·28	·133	1·0
O	13·673	·854	6·4

These data lead to a formula of about $Ba(C_{19}H_{31}O_3)_2$.

ι. Product of the Secondary Chemolysis with Barita and Caustic Soda in Succession.—On account of these unsatisfactory results, and from a fear that the salt might contain traces of undecomposed kephalin, it was again submitted to a barita chemolysis; but as it agglomerated into large masses, into the interior of which the barita had

little access, it was decomposed by treatment with hydrochloric acid and water. The free acid after solution in ether and distillation to dryness was dissolved in warm dilute caustic soda, a large excess of soda added, and the whole boiled seven or eight hours a day for several successive days. There was great frothing which could not be prevented, but any loss was obviated by allowing the froth to issue from the wide beak of the platinum retort employed, into a large beaker where it slowly subsided. From time to time the fluid which collected was returned to the retort. At the end there was obtained a brown turbid solution showing on agitation the silky clouds common in soap solutions. It was filtered as clear as possible by the vacuum method, and the solution precipitated with acetate of lead. The voluminous precipitate, after washing and drying, was extracted with ether, when it mostly dissolved, leaving, however, an amount of insoluble salt, probably stearate. The clear ether solution was treated with a concentrated solution of tartaric acid, the ethereal solution of acid separated from tartrate of lead, distilled to dryness, dissolved in a minimum of caustic soda, and precipitated with barium chloride. The barium precipitate was washed, dried, suspended in ether, and a solution was separated by decantation and filtration from a white insoluble salt which appeared much swollen in ether. The insoluble salt was analysed, but did not lead to any formula. The ethereal solution after concentration was precipitated by addition of a minimum of absolute alcohol and the precipitate analysed.

	Percentages.	÷At. Wgts.	÷Ba = 1.
C	58·55	4·878	34·64
H	8·45	8·45	60·01
Ba	19·29	·1408	1·00
O	13·71	·8569	6·08

leading to the formula $Ba(C_{17}H_{30}O_3)_2$.

The filtrate on examination was found to contain a small quantity of a kephalin-like body. The precipitate was therefore again dissolved and reprecipitated by a little alcohol, washed with absolute alcohol, and analysed :

	Percentages.	÷At. Wgts.	÷Ba = 1.
C	57·82	4·818	33·20
H	8·14	8·14	56·10
Ba	19·89	·1451	1·00
O	14·15	·8842	6·09

pointing to an empirical formula $Ba(C_{17}H_{28}O_3)_2$.

The acid obtained by decomposing this salt with hydrochloric acid is a dark-coloured viscid oil at the ordinary temperature, which is wholly soluble in alcohol, the solution not being decolorised by even large quantities of animal charcoal. On evaporation, or on addition of water, the acid separates from the alcohol in brown oily drops. On fusion with potash, no solid acid is obtained, but a brown acid which has all the properties of the original body, and gives a barium salt which contains about 19 per cent. of barium, and is soluble in ether.

Thus this acid gives none of the ordinary physical guarantees of purity, but the pertinacity with which it retains its composition and properties under the most varied and severe treatment, points distinctly to its chemical unity, while the quantity in which it occurs shows its radicle to be a principal ingredient in the kephalin molecle.

ϰ. Theory of the Chemical Constitution of the Kephalins.—According to the theories hitherto in vogue, kephalin may be regarded, considering its elementary composition and the products of its chemolysis, as a body in which two hydroxyls of the glycerin molecle are replaced by fatty acids, and in which the third hydroxyl is replaced by phosphoryl, which latter in its turn has one hydroxyl replaced by an ammonium base, thus :

$$C_3H_5 \begin{cases} \text{Fatty acid A.} \\ \text{Fatty acid B or C.} \\ HO(OPO)C_5H_{12}NO. \end{cases}$$

The whole of the phosphorus is apparently contained in the radicle of glycerophosphoric acid, and in no other form. This body is first obtained on chemolysis, but being somewhat unstable, it is not difficult in the presence of bases to decompose it further into glycerin and phosphate of the base used.

The whole of the nitrogen appears to be present as neurin ; the other bases which have been obtained being probably derived from neurin by secondary changes. This secondary decomposition would explain why the amount of the neurin obtained from a given weight of kephalin is less, sometimes much less, than the amount which theory would lead us to expect.

Of the fatty acids contained in kephalin, various barium, lead, and magnesium salts have been examined and analysed. It is highly probable that the acid contained in the main soluble in ether, barium, or lead salt, namely *kephalic* acid, belongs to a

series of acids containing at least one atom of oxigen more than the ordinary fatty acids. It is this fatty acid which impresses its peculiar character upon all the kephalins, and without its presence a phosphorised body constituted as above assumed does not seem to exhibit the properties of a kephalin. But the kephalins may vary as regards the second acid; in the principal kephalin this acid is *stearic;* but in certain kephalins which occur in subordinate quantity the second acid is either an acid of lower fusing-point than, though probably homologous with, stearic acid, or an acid not homologous with stearic, and giving a lead compound soluble in ether. The constitutional formula of the principal kephalin, or *kephalo-stearo-neuro-glycerophosphate* would thus be the following:

$$C_3H_5 \left\{ \begin{array}{l} C_{17}H_{28}O_3 \text{ (kephalyl)} \\ C_{18}H_{35}O_2 \text{ (stearyl)} \\ HO\,(OPO)\,C_5H_{12}NO \end{array} \right\} = C_{43}H_{81}NPO_9$$

A kephalin with palmityl, $C_{16}H_{31}O_2$, in place of stearyl would have the summary formula $C_{41}H_{77}NPO_9$; a kephalin with margaryl, $C_{17}H_{33}O_2$, would be $C_{42}H_{79}NPO_9$. If there were several homologous kephalic acids such as some analyses seem to indicate, then for a kephalyl of formula $C_{18}H_{30}O_3$ combined with either stearyl, or margaryl, or palmityl, the foregoing formulæ would have to be increased by CH_2 each, so that the most complicated kephalin might contain 44 atoms of carbon.

It will be seen that none of these hypotheses explain either the deficiency of hydrogen or the excess of oxigen in the various kephalins and their compounds which have been analysed. This discrepancy can only be eliminated by further researches carried on by the light of those given in the foregoing.

We have considered the kephalins as glycerides, in which three hydroxyls are substituted. It is, however, evident from the constitution of sphingomyelin, to be described below, and generalised in the introduction, that they may also be considered as *phosphatides,* or bodies held together by phosphoric acid, thus:

Phosphoric Acid.	*Kephalin.*
$OP \left\{ \begin{array}{l} HO \\ HO \\ HO \end{array} \right.$	$OP \left\{ \begin{array}{l} C_{17}H_{28}O_3 \text{ (kephalyl)} \\ C_{18}H_{35}O_2 \text{ (stearyl)} \\ C_3H_7O_3 \text{ (glyceryl)} \\ C_5H_{11}ON \text{ (neuryl)} \end{array} \right\} C_{43}H_{81}NPO_9.$

the neuryl replacing an hydroxyl in glyceryl, and being the

radicle which is the earliest to be detached by chemolysis. On this assumption the *kephalophosphoric acid* above described would have the formulæ:

$$OP \begin{cases} C_{17}H_{28}O_3 \\ C_{18}H_{35}O_2 \\ C_3H_7O_3 \end{cases} = C_{38}H_{70}PO_9.$$

3. Paramyelin: its Isolation, Analysis, and Compounds.

Paramyelin is a nitrogenised phosphatide, and is in the first stage diagnosed and separated by the solubility of its cadmium chloride compound in hot benzol, from which solution, filtered boiling, it is deposited on cooling. In this condition the compound is very voluminous and gelatinous, and is only with very great difficulty separated from the lecithin cadmium chloride compound which remains in solution in the cold benzol. The mechanical aids to this separation are two in number; one the cylindrical vacuum filter described, the other the use of very large volumes of benzol for the extraction of the soluble compound, the removal of the solution by the syphon from above the deposit, and very frequent repetition of this process. In this process the large volumes of benzol require many distillations for recovery; the deposition of the paramyelin compound requires time. But when once obtained free from matters soluble in cold benzol, the compound behaves with great precision. It is, whilst moist with benzol, washed with spirit, which dissolves any colouring-matter, and it is well to repeat this washing by shaking the compound in a bottle, etc., until the supernatant spirit is colourless. The compound thus obtained is soluble in boiling spirit, and deposited on cooling; it is easily decomposed by hydrothion in spirit, and the hot solution yields on cooling white crystallised paramyelin hydrochlorate. This by re-crystallisation or washing loses its acid easily, and free crystallised paramyelin remains.

A specimen of paramyelin cadmium chloride compound obtained from the extracts of human brain by the process described under lecithin, but without the previous purification by lead, soluble in boiling, insoluble in cold benzol, dried at 100°, gave on analysis 13·70 per cent. Cd, 3·59 per cent. P, 9·68 per cent. Cl (this requires 15·27 per cent. Cd, whereas only 13·70 per cent. Cd were found, total $CdCl_2$ calculated from Cl = 24·95 per cent.), 1·69 per cent. N, 47·19 per cent. C, and 7·94 per cent. H.

These data exhibit the following ratios :

$$P : N = 1 : 1{\cdot}06$$
$$P : CdCl_2 = 1 : 1{\cdot}20$$
$$N : CdCl_2 = 1 : 1{\cdot}13$$

$$\left.\begin{array}{r}\text{If } CdCl_2 \text{ be calculated from Cl} = 24{\cdot}95 \\ \text{then the organic molecle is} = 75{\cdot}05\end{array}\right\} = 100$$
$$\left.\begin{array}{r}\text{If } CdCl_2 \text{ be calculated from Cd} = 22{\cdot}39 \\ \text{then the organic molecle is} = 77{\cdot}61\end{array}\right\} = 100$$

These relations thus show a slight excess of nitrogen over the relation $N : P = 1 : 1$, and a more important excess of cadmium chloride over the presumable relation $N : CdCl_2 = 1 : 1$. But considering that the compound is a first product, the relations are satisfactory, as showing the firmness of the compound. In the following I give a comparison of this salt with the paramyelin salt from ox first described.

Summary of Human Paramyelin Cadmium Chloride.

	Percents.	
C	47·19	75·05
H	7·94	
N	1·69	
P	3·59	
O	14·64	
(Calcd.) Cd	15·27	24·95
(Found) Cl	9·68	

Summary of Ox Paramyelin Cadmium Chloride.

	Percents.	
C	49·288	78·725
H	8·299	
N	1·598	
P	3·396	
O	16·144	
(Calcd.) Cd	13·020	21·275
(Found) Cl	·255	

Computation of the Organic Moleclcs of these Salts.

		Percents.	÷ by At. Ws.	÷ P=1.		Percents.	÷ At. Ws.	÷ P=1.	
408	C	62·87	5·239	34	C	62·607	5·217	37·5	456
68	H	10·58	10·58	68	H	10·541	10·541	75·8	76
14	N	2·25	0·160	1·03	N	2·029	0·144	1·0	14
31	P	4·78	0·154	1	P	4·313	0·139	1·0	31
128	O	19·50	1·24	8	O	20·510	1·281	9·2	144
649									721

The percentages of carbon and hydrogen are practically identical in both organic molecles. Other data show distinctly that the salt is not, and does not contain, either amidomyelin or sphingomyelin. The absence of lecithin follows from the solubility in cold benzol of its cadmium chloride compound. The data, however, do not show that the compound is unitary, and

does not contain two or more similar principles (paramyelins). They also do not show the exact composition of the organic molecle, for, as we know from a vast amount of experience, this can only be ascertained with the aid of several compounds, and of the decomposition products of the principle under the influence of chemolytic agents. For the study of these relations the past has afforded no time or opportunity, and it must therefore be left to the future.

Preparation of Free Paramyelin from the Cadmium Chloride Compound.

The $CdCl_2$ salt of human paramyelin was suspended in spirit, and treated with H_2S, at first at the ordinary temperature, later on at 75° in the water-bath, until it was completely decomposed. The filtrate, on standing and cooling, deposited white crystallised paramyelin. The crystals were rhombic and hexagonal plates of microscopic dimensions. They were collected on a filter, washed, pressed, recrystallised from spirit to remove a trace of colour and the rest of the hydrochloric acid, and dried in vacuo. On analysis they gave 4·31 per cent. P, and 2·06 per cent. N.

These two analyses show that N : P = 1·00 : 1·00.

The atomic weight of paramyelin as deduced from the percentage of phosphorus is 688; as deduced from nitrogen, 666; mean, 677 (for the free body). The atomic weight of paramyelin (human) as deduced from the organic molecle in the $CdCl_2$ salt is 649; that for ox paramyelin combined in the same manner is 721; mean 680. These two means are practically identical.

The question now arises whether this phosphatide contains glycerol or not, neurin or not; these questions can be answered by chemolysis only.

Paramyelin Cadmium Chloride (Ox).—$C_{38}P_{75}NPO_9,CdCl_2$. *From Ox-buttery after Kephaloidin.*

When the buttery matter dissolved in ether had been precipitated by alcohol and the kephaloidin been removed, the mother-liquor on standing deposited some secondary kephaloidin and cholesterin. These were filtered off; the liquid was precipitated with $CdCl_2$; the precipitate was washed with alcohol, and pressed; it was next extracted with ether (which dissolved a small quantity of kephaloidin $CdCl_2$) until pure, dried, and analysed. The result of the analyses showed that the body was a $CdCl_2$ compound.

The organic molecle amounted to 77·49 per cent.; the $CdCl_2$ to 22·51 per cent.

Treatment with Benzol.—It was found that the compound was entirely soluble in boiling benzol, and deposited a portion on cooling which was white and voluminous. Another portion remained dissolved in the cold benzol. The deposit was isolated by filtration, dissolved once more in boiling benzol, and was deposited as a swelled gelatinous mass; from this benzol was drained by blotting-paper, and the residue was dried.

Paramyelin Cadmium Chloride, $C_{38}H_{75}NPO_9,CdCl_2$.—Insoluble in cold benzol. The analyses were carried out in the usual manner, but the Cd was not estimated.

Summary:

		Percents.	
	C	49·288	78·725 (C, H, N, P, O)
	H	8·299	
	N	1·598	
	P	3·396	
Hypothetical -	O	(16·144	
Hypothetical -	Cd	(13·020)	21·275 (Cd, Cl)
	Cl	8·255	
		100·000	100·000

Computation of Organic Molecle:

	Percents.	÷ by At. Wgts.	÷ by P = 1.
C	62·607	5·217	37·5
H	10·541	10·541	75·8
N	2·029	0·144	1·0
P	4·313	0·139	1·0
O	20·510	1·281	9·2
	100·000		

The formula $C_{38}H_{75}NPO_9$ gives an atomic weight of 720, but the atomic weight calculated from the $CdCl_2$ is only 677; 720 + 183 ($CdCl_2$) = 903 requires 20·2 per cent. $CdCl_2$. There is, therefore, still an irrationality between the chloride and the organic molecle, in the sense of the metallic salt being in excess.

4. Myelin: its Isolation, Analysis and Compounds.

General Definition of Myelin.—The leading features of the principle here to be described will distinguish it with great precision from all similar matters. When freshly obtained it is white like

bleached ivory, but when kept for some time it becomes a little yellowish and waxy. It crystallises, from ether or absolute alcohol solution on slow evaporation, in curved needles and scales of a rhombic ovoid shape, which are well seen under the microscope with a power of × 400. When it is in minute crystals it remains powdery even after drying, but when drying in body after deposition from alcohol and washing by ether, it becomes transparent and waxy, cuts like dry walnut kernel, and when dry can be powdered. The powder is perfectly white. It swells and emulges with water, particularly on the application of heat, in the manner defined for all phosphorised cerebral principles. The solution iridesces bluish-white from polarisation of the minute particles. This solution gives the reactions to be described. It dissolves in hot alcohol abundantly, and is deposited on cooling in white tufts, granules, and masses of peculiar appearance, and on slow evaporation in crystalline needles. The alcohol retains little myelin in solution when cold, and gives no precipitates with $CdCl_2$ and $PtCl_4$. It dissolves very sparingly in hot ether, and is almost immediately deposited from this solution when its temperature sinks. It is less soluble in cold ether than in boiling. It contains more than three per cent. of phosphorus. With Pb acetate and ammonia it gives a white salt, which is insoluble in alcohol and ether, and contains an atom of lead. Myelin is consequently a dibasic acid.

Modes of obtaining Myelin.—It can be obtained directly, without the intervention of precipitants, from the cold alcohol extracts of white matter, by concentration and cooling, redissolving the precipitate, and letting the solution stand for a long time in the cold, when myelin is deposited crystalline. After isolation it has to be washed with a little ether, combined with lead, and freed from sphingomyelin by boiling spirit.

The ether extracts of white matter may be precipitated by alcohol, the precipitate emulged with water, treated with lead acetate and ammonia, washed, and extracted with hot alcohol and ether in succession, when ultimately white myelin lead, of the formula to be given, remains insoluble in these agents. The lead salt decomposed by H_2S in water, and the precipitate extracted with hot alcohol, yields white myelin; or the lead compound may be decomposed in hot spirit by hydrogen sulphide.

Differences and Separation from other Cerebral Principles.—Myelin

can be separated from kephalin and kephaloidin and allied bodies by the operator using the peculiarity of its being *very little soluble in cold ether*, in which these bodies and their compounds are easily soluble. The solutions should always be exposed to a very powerful freezing mixture, and filtered through a filter and funnel surrounded with freezing mixture. From *lecithin* myelin can be separated by the lead process.

Myelin can be separated from the *cerebrosides* by much boiling alcohol, in which both are largely soluble, but the cerebrosides are almost insoluble in cold alcohol, in which, therefore, myelin would remain dissolved on cooling more readily. But the lead process is preferable, and necessary in any case as a means of precipitation.

An absolute separation of myelin from the other phosphorised principles is best effected by the processes employing lead acetate, and from cerebrosides (as obtained by alcohol process) by extraction with boiling alcohol and separation after cooling.

No phosphatide, however similar in bearing to myelin, should be assumed to be myelin before it has been in combination with lead, and been found insoluble as lead salt in boiling alcohol and in ether.

Myelin Lead.—$C_{40}H_{73}PbNPO_{10}$. The ether-solution from white matter, after exhaustion by freezing, was precipitated by alcohol; the bulky precipitate was filtered, washed, and dried in vacuo, and during this process repeatedly pounded in a mortar. It was now swelled in water, and subjected to dialysis; it formed a thick, slimy, gummy or starch-like emulsion, in which many small crystals of cholesterin formed. The addition of watery Pb acetate produced a dense curd, which separated easily from fluid; it was placed on a cloth filter and allowed to drain away its mother-liquor. The precipitate was placed in alcohol and warmed, whereby little else but water was extracted (one litre alcohol left on evaporation to dryness a little brown matter). More warm strong alcohol now extracted much cholesterin. Hot boiling absolute alcohol extracted *much* cholesterin and a little yellow smeary lead salt. The insoluble part was soft, waxy, but on cooling granular. It was now placed in ether, whereby a yellowish fluorescent lead salt of kephalin was extracted. This latter salt was precipitated by absolute alcohol, deposited as a yellowish oily body, which became hard on standing. This has been treated under kephalin. *A white pulverulent salt* remained

insoluble in the ether, was thoroughly washed with ether on the filter, also shaken with ether in a bottle, and again washed on the filter. It shrunk much on drying. It was insoluble in benzol.

Substance dried at 100° C. gave, on analysis, data which are arranged in the following summary of analyses and theories :

	Percentages.	÷ by At. Wgts.	÷ by Pb = 1.	÷ by N = 1.	÷ by P = 1.
C	50·88	4·240	44·63	41·56	40·38
H	7·89	7·890	83·05	77·35	75·13
Pb	19·76	0·095	1·00	0·93	0·90
N	1·44	0·102	1·07	1·00	0·97
P	3·28	0·105	1·10	1·02	1·
O	16·75	1·046	11·01	10·25	9·96
	100·00				

The organic body in the salt = 100 − 19·76 = 80·24.

Percentages of elements found in organic body and theories :

		÷ by At. Wgts.	by N = 1.	÷ by P = 1.
C	63·409	5·2840	41·24	40·09
H	9·833	9·8330	76·76	74·60
N	1·794	0·1281	1·	0·90
P	4·087	0·1318	1·02	1·
O	20·874	1·3046	10·18	9·89
	99·997			

There are thus arguments at hand for atomic weights with from 40 atoms to 44 atoms of carbon ; but the combined metals and salts are perhaps less to be relied upon for atomic weight determinations of the phosphorised principles than the constitutional elements P and N. These latter, therefore, prevail in my opinion as determinants, particularly as they agree well with each other. I therefore accept $C_{40}H_{73}NPO_{10}$ as the formula of the body combined with lead, and adding 2H in the place of Pb, the formula of the free body will be $C_{40}H_{75}NPO_{10}$.

Theory of			Found.
Elements.	At. Wgts.	Percentages.	
40 C	480	63·15	63·409
75 H	75	9·86	9·833
1 N	14	1·84	1·794
1 P	31	4·07	4·087
10 O	160	21·05	20·874
	760		

Decomposition of Pb Salt by H_2S.—A portion was decomposed by H_2S while suspended in ether. The ethereal filtrate from the PbS deposited a white flaky matter on being shaken, which increased in quantity on standing. It was allowed to go to dryness spontaneously, and left an abundant white residue, which was soft, and smelled peculiarly. It fused above 100°, was perfectly fused about 125° to 130°, and on cooling was quite solid again at 100°. On being heated further it cracked and spirted, then gave off strong-smelling fumes, burnt with a white luminous flame, and left a charcoal difficult to incinerate. Fused with nitre and soda, and the fused mass dissolved in HNO_3, the tests for lead gave negative results, but the tests for P_2O_5 gave evidence of abundance; so that the H_2S treatment removed all the lead.

The supposed PbS, when heated, fused, and gave off carbonaceous vapours, and behaved in such a manner as to indicate that it did yet contain much organic matter.

The entire quantity of finely-powdered lead salt was now placed in absolute alcohol, and decomposed with H_2S while being heated in a water-bath, filtered hot, and extracted with hot alcohol as often as was necessary to effect complete exhaustion. The alcohol extract, on cooling, deposited a crystalline mass. This was recrystallised from absolute alcohol, when a tendency to stearoconotise became evident in the deposit; but all ultimately dissolved with the aid of hot ether, and the first purest portion of crystals was analysed. Dried at 100° C, they became coloured on surface.

Summary and Computation:

	Percents.	÷ by At. Wgts.	÷ by P=1.
C	62·651	5·221	39·0
H	10·340	10·340	77·1
N	2·000	0·142	1·
P	4·170	0·134	1·
O	20·829	1·301	9·7

The isolated myelin thus exhibits the formula $C_{39}H_{77}NPO_9$.

B. SUBGROUP OF DINITROGENISED MONOPHOSPHATIDES. N : P = 2 : 1.

1. AMIDOMYELIN: ITS ISOLATION, ANALYSIS, AND COMPOUNDS.

Amidomyelin is met with in certain precipitates obtained from brain extracts by the agency of cadmium and platinum chloride. These precipitates are, as regards crystalline appearance and bearing

towards solvents, seemingly homogeneous; but on elementary analysis they show an irrationality between the phosphorus and nitrogen, which in not a few cases assumes the proportion of P : N = 2 : 3, all other elements being present in nearly the same average atomic proportions as those in which they are found in phosphatides in which P : N = 1 : 1. I explain these variations of the nitrogen as due to the presence of a compound in which P : N = 1 : 2. After the discovery of *apomyelin*, and lately of *sphingomyelin*, in both of which principles P : N = 1 : 2, I isolated *amidomyelin* by the processes which have already been partially described under the chapter relating to lecithin. These processes had to be guided by incessant quantitative elementary analysis by which to control the progress and direction of the purification of the principle sought to be isolated. It was found that differentiating solvents and combinants were the principal means for effecting this isolation and purification, and that so-called fractional crystallisation and recrystallisation were only of subordinate value. No diagnostic value was found to be attached to so-called uniform crystalline or crystallised appearances; for a great number of chemically similar or dissimilar bodies would crystallise in such a manner as to make the impression of homogeneity upon the eye, while their diversity could be quickly and incontrovertibly proved by appropriate chemical reagents.

Process for the Isolation of Amidomyelin.

The buttery matter from human or bovine brains is dissolved in hot spirit, and to the solution an ammoniacal solution in spirit of lead acetate is added as long as a precipitate is produced. This precipitate is removed by filtration on a funnel heated by steam. It contains kephaloidin and myelin as lead salts; lead salts of phosphatides, free from nitrogen, and some lead salts of cerebrinacides, the latter in small quantity. The filtrate deposits on cooling a mixture of cholesterin with lead salts, particularly of myelin and cerebrinacides, and other phosphatides in the free state, amongst them some amidomyelin. The clear filtrate is mixed with a spirituous solution of cadmium chloride as long as a precipitate is produced: an excess of cadmium solution is then added, and the mixture is allowed to stand for the precipitate to contract and settle.

The mixture of cholesterin, lead salts, and other phosphatides,

is again boiled with spirit and allowed to become cool without having been filtered while hot. The liquid is filtered from the insoluble and crystallised matter, and in its turn treated with cadmium chloride solution in the manner stated for the first solution. In this manner the cholesterin and lead salts mixture is extracted with spirit as long as the mother-liquors give precipitates with cadmium chloride. The last extracts are the richest in amidomyelin. The cadmium chloride precipitates are all united, washed with spirit by decantation until the washings are colourless, exhausted with ether by decantation, dried in vacuo over oil of vitriol, powdered, and subjected to the benzol process, whereby they are separated into the three different compounds which have been described under lecithin. The compound insoluble in boiling benzol is the amidomyelin dicadmium chloride compound. When the cadmium precipitates are not thrown together, but treated separately by the benzol process, it is observed that those obtained from the earliest spirit solutions contain only little amidomyelin, while this ingredient gradually increases in quantity until in the cadmium precipitate obtained from the ninth or tenth solution there is found as much as 40 per cent. of the salt insoluble in boiling benzol.

Amidomyelin Dicadmium Chloride Compound, Insoluble in Boiling Benzol from Ox buttery after Lead Process.

Synopsis of Analyses:

	(1.)	(2.)	(3.)	(4.)	(5.)	(6.)	(7.)	(8.)
C	43·78	43·87	—	—	—	—	—	—
H	7·68	7·72	—	—	—	—	—	—
N	—	—	2·38	2·55	2·38	—	—	—
P	—	—	—	—	—	2·57	2·58	—
Cd	—	—	—	—	—	—	—	17·99
Cl	—	—	—	—	—	—	—	11·42

Mean of Analyses and Theory of Formula.

	Percents.	÷At Wgts.	÷P=1.	÷N=1.	Theory.		
C	43·825	3·652	43·73	42·	C_{44}	528	839
H	7·70	7·70	92·2	88·	H_{92}	92	
N	2·43	0·1735	2·07	2·	N_2	28	
P	2·59	0·0835	1·	0·96	P	31	
O	14·045	0·8775	10·5	10·	O_{10}	160	
Cd	17·99	0·1606	1·92	1·84	Cd_2	224	366
Cl	11·42	0·321	3·84	3·70	Cl_4	142	
	100·000					1205	

$C_{44}H_{92}N_2PO_{10}(CdCl_2)_2$	requires	30·37	per cent.	$CdCl_2$.
	found	29·41	,,	,,
$C_{44}H_{92}N_2PO_{10}CdCl_2$	requires	17·90	,,	,,

Preparation of Free Amidomyelin from the Cadmium Chloride Compound.

(*a*) *By the Hydrothion Process.*—The cadmium chloride salt described in the foregoing is finely powdered, suspended in spirit, and the mixture is saturated with hydrothion at the ordinary temperature. It is then heated in a water-bath until the spirit boils, while the introduction of the sulphuretted gas is continued until the cadmium is all transformed into the yellow sulphide. When a filtered sample of the solution is no longer altered by hydrothion, the whole is isolated by filtration on a heated funnel. On cooling it crystallises, the quicker the more concentrated it is. After twenty-four hours' standing the crystals are collected, washed with spirit and pressed, redissolved in a minimum of spirit (in which, before dissolving, they melt into an oil), and again allowed to crystallise. This process is repeated until crystals and mother-liquor are both perfectly colourless. In this process there is the danger that some of the amidomyelin is decomposed under the influence of the four molecles of hydrochloric acid which are set free by the decomposition of the cadmium chloride. This chemolysis may yield some fatty acid, which may remain mixed with the amidomyelin, and is difficult to remove. In consequence, the carbon and hydrogen of the free body may be found higher than the theory derived from the salt. The free body also retains some hydrochloric acid—in the first instance, less than 1 per cent. (found 0·93 per cent. Cl)—which diminishes to a trace by repeated crystallisation from spirit. But to remove this hydrochloric acid entirely requires a circumstantial process and the employment of silver oxide or mercuramin, and, again, hydrothion and frequent recrystallisation.

(*b*) *By Dialysis.*—The cadmium compound is suspended in water, and placed in a corrugated piece of vegetable parchment, folded like a plaited filter, and placed inside a funnel. The funnel is closed with a cork, or some kind of tap, at its lower aperture, and the space between the funnel and parchment is filled with distilled water. This is renewed as long as it contains cadmium chloride, indicated by sulphide of ammonium and nitrate

of silver. *The amidomyelin is at last found to be completely dissolved in the water*, and the solution can be filtered clear through the densest filtering-paper. When this solution is gently warmed, it sets into a jelly. This latter may be evaporated on the water-bath, with constant stirring, to near dryness. The residue is dissolved in hot spirit, the solution treated with hydrothion to remove a trace of cadmium, and allowed to cool and stand. White amidomyelin crystallises, to be purified by recrystallisation, etc., as above described.

(*c*) *From the Acid Mother-liquors*, filtered from the crystallised amidomyelin, the hydrochloric acid is removed by mercuramin added in fine powder, with stirring and warming. When the powder does not any longer change colour, but retains its canary-yellow tint, all the acid is precipitated. The solution is filtered warm, and, yet warm, treated with a little hydrothion to remove the trace of mercuramin which is dissolved in the hot spirit. It is then concentrated, and allowed to crystallise ; or if coloured, it is mixed with cadmium chloride solution, and the washed cadmium chloride compound is treated anew as above described.

Properties of Amidomyelin.

Amidomyelin crystallises in snowy-white microscopic plates and needles, arranged in stars and disposed in irregular masses. They dry in vacuo over oil of vitriol to a perfectly white mass, which is easily powdered, and can be dried in the water-oven below 100°. With sugar and oil of vitriol amidomyelin gives the purple of Raspail's reaction rather quickly and deeply ; it is at present not known whether the reaction is due to the presence of the oleyl, cholyl, or sphingosyl radicle in the molecle of the amidomyelin. The remarkable solubility of the freshly dialysed amidomyelin in cold pure water, and insolubility in slightly warmed water, must again be pointed out. The change which it undergoes when its watery solution is warmed is permanent : the jelly produced by warmth does not redissolve on cooling. This phenomenon is of great importance in the study of the functions of the immediate principles in the brain ; it is calling for further investigation, and comparison with the bearing of the other phosphatides under similar conditions. One of these, not yet accurately identified, has the property of being slimy and diffused and unfilterable in cold water, while becoming hard and contracted in boiling water,

so that the water can be filtered off. This substance, with fresh cold water, gradually resumes the slimy greatly hydrated condition.

Theory of Amidomyelin (Ox) as deduced from its Cadmium Chloride Compound, and Comparison with the Theory of Sphingomyelin (Apomyelin).

Taking the organic molecle from the analysed $CdCl_2$ salt, with 29·42 per cent. $CdCl_2$, namely 70·59 per cent., and calculating elements for 100, we get—

	For Amidomyelin—				Sphingomyelin gives— $C_{52}H_{104}N_2PO_9 + H_2O$.		
	Percents.	÷ At. Wts.	Atoms.	At. Wts.	Percents.	Atoms.	
C	62·15	5·179	44	528	68·37	51·86	624
H	10·93	10·93	92	92	11·29	107·52	106
N	3·37	0·24	2	28	2·96	2·	28
P	3·65	0·117	1	31	3·24	1·	31
O	19·90	1·24	10	160	17·14	10·2	160
				839			949

Diagnosis and Separation of Amidomyelin from Sphingomyelin.

Elementary analysis, with special regard to the relations between N, P, and C, is as yet the only means of establishing a diagnosis between these two principles. It is satisfactory that in the first steps of brain extraction they separate in the main; amidomyelin remains with paramyelin and lecithin, while sphingomyelin remains with the cerebrosides and cerebrinacides. But the analyses of sphingomyelin in early stages of purification make it probable that it is mixed with a mononitrogenised phosphatide as insoluble in cold spirit as itself, a body closely resembling or identical with paramyelin. From paramyelin amidomyelin is easily separated by the benzol process applied to cadmium chloride salts. This process will therefore aid to separate paramyelin from sphingomyelin also. But it has not yet been possible to exactly fix the bearing of sphingomyelin and its cadmium chloride salt towards benzol, nor to establish its absolute diagnosis and absolute separation from amidomyelin when both occur in a state of admixture with each other. Neither has there been time or material for the study of the chemical constitution of amidomyelin by means of the chemolytic method, which I have shown

abundantly in the course of these researches to be the only means for obtaining precise final knowledge regarding the atomic composition and weight of these marvellous ingredients of the brain, nerves, and protoplastic centres.

2. Amidokephalin, its Isolation, Analysis and Compounds.

Amidokephalin.—The following preparation was made from a specimen of crude kephalin which had not undergone the purifying process with water, filtration, and hydrochloric acid. It had been thrice precipitated from ether by alcohol, and when last dissolved in ether, had stood during twenty-four hours in ice, to deposit traces of myelin and sphingomyelin.

Summary of Analyses of the Free Kephalin employed in this Experiment.

		÷by At. Wgts.	P as 1.	P=3.
C	59·51	4·96	42	127
H	9·31	9·31	79	238
N	2·73	0·195	1·6	4·98
P	3·64	0·117	1	3
O	24·81	1·550	13	39·7
	100·00			

It will thus be seen that all the elements are in proportions required by pure kephalin, except the nitrogen, which is six-tenths of an atom too high, or amounts to nearly five atoms, if three atoms of phosphorus are assumed.

If, as is probable from analogy with amido- and sphingomyelin, there are kephalins containing amidated acids, as proximate conjugated compounds, which amidated acids have also now and then been found in the chemolyses of kephalin, then it might be supposed that out of six fatty acid radicles in three molecles of kephalin, two fatty acid radicles were amidated.

The facts are expressed by the formula—

$$\left.\begin{array}{l} C_{42}H_{80}N_2PO_{13} \\ C_{42}H_{79}N\,PO_{13} \\ C_{42}H_{80}N_2PO_{13} \end{array}\right\} = C_{126}H_{239}N_5P_3O_{39}.$$

The preparation was proved to be free from sulphur by special analysis. Some anomalies in details evidently do not yield to hypothesis.

Transformation of this Preparation into Lead Salt.

The specimen of kephalin was therefore again frozen in ether, and deposited a vestige of white matter; the clear solution was then poured into absolute alcohol containing lead acetate; the precipitate was filtered, washed, suspended in absolute alcohol, warmed to 45° to 50°, filtered hot, to extract soluble matters. No deposit occurred in this alcohol on cooling. The lead salt was dried in vacuo. powdered, and found highly electric. It was suspended in and extracted with ether.

Summary of Analyses:

C	44·14				
H	6·583				
N	1·07				
Pb	27·86	27·49	26·10	26·54	Mean = 26·99
P	2·97	3·09			Mean = 3·03
O	18·25				
	100·00				

Calculation shows that in this compound the lead does not stand in any stoichiometric relations to any one element. It is in excess by about one-fourth over the quantity it should be if one atom of kephalin were combined with one atom of lead. Deducting the lead, and calculating the organic matter, *i.e.* 73·01 as 100, we get—

$\bar{K}$ in Pb salt.		Free $\bar{K}$ before.	Pure $\bar{K}$ gives.	Theory requires.
C	60·5	59·51	60·00	60·28
H	9·1	9·31	9·39	9·44
N	1·46	2·73	1·68	1·67
P	4·06	3·64	4·27	3·70
O	25·02	24·31	24·66	24·88
	100·00	100·00		

The ether and lead treatment, the combination and purification thereby effected, have therefore brought this kephalin much nearer to the composition of the purest.

3. SPHINGOMYELIN.—TYPE OF THE DIAMIDATED PHOSPHATIDES, WHICH CONTAIN NO GLYCEROL: ITS ISOLATION, ANALYSIS, CHEMOLYSIS, AND COMPOUNDS.

In chemical researches which have to deal with the separation of the ingredients of complex mixtures, the difficulties generally rise with the atomic weights of the bodies to be treated. Of

this rule, sphingomyelin, a body the atomic weight of which is probably one of the highest after those of the albuminous class, has furnished a striking confirmation.

Sphingomyelin is the principal, but not the only, phosphorised ingredient of the so-called cerebrin mixture, which remains when white matter is exhausted by ether. Out of this mixture there have been isolated entire series of immediate principles; first and best studied, the *cerebrosides*, with *phrenosin* as their chief; secondly, the *cerebrinacides*, which contain more oxygen than the former, and combine with lead; thirdly, bodies containing sulphur as an essential ingredient, hence termed *cerebrosulphatides*. To these we have now to add the description of *peculiar phosphatides*, and certain nitrogenised substances free from phosphorus, which may be termed *nitrogenised fats* or *amidolipotides*. The processes described in the following are those which actually led to the discovery by which they were rewarded. But it is probable that, after a complete knowledge of the properties of the newly discovered bodies has been obtained, these processes may be much simplified and shortened.

Process of separating apparently homogeneous Crystallised Bodies from the Alcohol used for the separation of the Cerebrosides; which Bodies will be shown to be Mixtures by Reagents.

After the removal of phrenosin and kerasin by crystallisation from absolute alcohol frequently repeated, there remained alcoholic solutions, which made no further deposit on standing. These were distilled to a small bulk, and deposited a white crystallised matter. (An attempt to separate anything out of the matter by benzol proved abortive; cold benzol extracted but little, on application of heat all dissolved, and on cooling the mixture set into an unmanageable jelly. The benzol was therefore distilled off, and the matter treated anew with spirit.) The crystalline matter was dissolved in hot spirit of 85 per cent. strength, and an alcoholic solution of lead acetate was added, after this a slight excess of ammonia. The cerebrinacides contained in the body were thus precipitated as lead salts. From these the solution was separated hot by filtration. After cooling, the alkaline mother-liquor was separated from the crystalline deposit. The washed crystalline mass was dissolved in hot spirit, and left much matter in a fused state (second lead stearoconote). A part

of the matter being yet insoluble lead compound should remain undissolved; but when fused it encloses a quantity of soluble matter, which then remains inaccessible to the spirit. It has been found useful to emulge the crystalline matter with some water before heating it with spirit. This resolution in hot spirit and recrystallisation are repeated four times, or until no lead salt remains insoluble, and until the product is uniformly white, and crystallised in stars and rosettes of needles, clearly visible under the microscope. It is dried in vacuo.

Properties of the Product.—With water it forms a permanent jelly or paste like starch pap. With sugar and sulphuric acid it gives an immediate purple reaction; with sulphuric acid alone it becomes thoroughly purple. Heated to between 90° and 100° in a water-oven, it becomes a little soft and a little coloured; when cold, it becomes again pulverisable. It fuses at about 150°, assuming a brown colour. It yields to absolute ether-alcohol a considerable amount of matter, which crystallises in the original rosettes and dichotomically branched masses, like lycopodium.

Preliminary Quantations of Elements.—Synopsis of Results.

Found in 100.		÷ At. Wgt.	÷ P = 1.
C	58·81	4·900	68·05
H	11·55	11·55	160·40
N	2·63	0·188	2·61
P	2·24	0·072	1·
O	24·77	1·548	21·50

These data show that the body, though crystallised as described, was yet a mixture. Its principal ingredients were *sphingomyelin*, in which P : N = 1 : 2, and *kerasin*, which latter mainly explains the excess of carbon and nitrogen and attached water, as will be shown below.

Comparison with this Rosette or Lycopodium-like Body of a similar Body obtained from the Cerebrin Mixture by Ether, together with the Kephalin, etc.

The ether extracts from white matter containing all kephalin, myelin, cholesterin, etc., were concentrated and mixed with an alcoholic solution of lead acetate. Kephalin and myelin became insoluble as lead salts. When the precipitated matters were boiled with spirit, and the filtrate was allowed to cool, cholesterin, together with a lead compound and the new body were deposited.

The deposit was isolated, dried, and extracted with ether not in excess. Cholesterin dissolved, while the lead salt and new body remained undissolved. The latter mixture was treated with boiling alcohol, which dissolved the new body, and deposited it on cooling in crystalline rosettes, while the lead salt remained undissolved as a fused mass. By frequent recrystallisation of the new body as long as it left any fused lead-compound, it was at last obtained quite free from lead and white.

Chemical and Physical Properties.—White crystalline mass. Becomes soft at 90°, without loss of water. Gives Raspail's reaction with and without sugar, from which the presence of a cerebroside may be inferred. The lead compound gave the Raspail reaction with and without sugar, if at all, very indistinctly.

Analyses of the second Rosette Body.—Synopsis of Results.

	Percents.	÷At. Wgt.	÷P=1.
C	58·67	4·889	119
H	11·18	11·180	272
N	2·78	0·198	4·82
P	1·33	0·041	1·
O	26·04	1·627	39·5

The crystallised body just analysed is a mixture of a dinitrogenised phosphatide, *sphingomyelin*, with *a cerebroside*, as was clearly shown by the application of reagents. But the nature of the cerebroside in this case was not ascertained as in the former case.

Isolation of Sphingomyelin by Cadmium Chloride Process.—Many attempts (of which only two have been described in the foregoing) having been made to isolate a constant product by mere crystallisation with solvents, without success, the cadmium chloride process was again adopted. The reagent was mainly applied to cold alcoholic solutions, and had the following effect. A precipitate of $CdCl_2$ salt ensued immediately. If this was filtered off quickly, a second more gelatinous precipitate fell, consisting of almost pure kerasin. The $CdCl_2$ salt also contained yet some kerasin. From this it was separated by boiling spirit; the $CdCl_2$ salt was deposited mainly above 28°, the kerasin entirely below 28°, and on long standing provided that the amount of kerasin did not rise above 1 part in 321 parts of spirit. Of the $CdCl_2$ salt, spirit retains less than a half per cent. (weight in volume) in solution. It is probable that the body or bodies which combine

with $CdCl_2$ (sphingomyelin and other phosphatides) on the one hand, and kerasin on the other hand, keep each other in solution by some attraction which they have for each other, in which sphingomyelin acts as base, kerasin as acid, the result being a kind of salt which is more soluble in spirit than each of its components by itself. The alcoholic solution from which the $CdCl_2$ salt and kerasin have been precipitated, must be allowed to stand long, and be repeatedly concentrated, to remove all kerasin. It then yields a precipitate with platinum chloride and hydrochloric acid, which is a compound of a phosphorised and nitrogenised body, *assurin*, with the reagents employed. The mother-liquor yields *krinosin and bregenin*, nitrogenised matters free from phosphorus, and belonging to the new class of *amidated lipotides* or *nitrogenised fats*, as will be fully described lower down.

Gradual Purification of the $CdCl_2$ Salt of Sphingomyelin by recrystallisation from boiling Spirit and Extraction with boiling Ether.

In the course of these processes it was found that the precipitates might contain varying quantities of $CdCl_2$, and that these might correspond to several compounds. Sphingomyelin might combine with one or two molecles of $CdCl_2$; the second phosphatide might combine with one molecle of $CdCl_2$ only, being a mononitrogenised body, probably paramyelin. The $CdCl_2$ might also be depressed by the admixture of kerasin. It was also found that the $CdCl_2$ would shift, so as to increase in one fraction, while diminishing in another of a previously unitary preparation.

The changes of these salts in general, and the extraction of the kerasins and other admixtures in particular, were followed analytically. Thus a CdCl salt gave 5·97 per cent. Cl and 10·89 per cent. Cd = 16·86 per cent. $CdCl_2$. The 5·97 per cent. Cl require in theory 9·41 per cent. Cd = 15·38 per cent. $CdCl_2$. The salt here obtained was the one with one molecle of $CdCl_2$; sphingomyelin with 51 carbon atoms and $CdCl_2$ postulates 16·4 per cent. $CdCl_2$; the body with 53 carbon atoms requires 15·98 per cent. $CdCl_2$.

Another salt from ox cerebrosides mother-liquor of recrystallisation was a mixture of sphingomyelin $CdCl_2$ in which N : P = 2 : 1, and of a phosphatide $CdCl_2$ salt, in which N : P = 1 : 1, probably paramyelin. The salts were crystallised from hot spirit above 30°, and recrystallised until free from kerasin.

They contained 13·14 per cent. Cd, 3·24 per cent. P, and

9·12 per cent. Cl, 2·28 per cent. N, 50·70 per cent. C, and 9·08 per cent. H.

The chlorine found in analysis (1) postulates 14·3 per cent. Cd. There is, therefore, a slight deficiency of this element. The organic molecle has therefore to be calculated by deducting the latter larger figure for Cd, namely, 14·3 per cent., thus:

$CdCl_2$ - - -	23·42	per cent.
Organic molecle -	76·58	,,
	100·00	

This quantity of $CdCl_2$ indicates that the mixture contains sphingomyelin monocadmium chloride with sphingomyelin dicadmium chloride, bodies which will be discussed more explicitly lower down. The nitrogen is to the cadmium chloride in such a proportion that the basicity of sphingomyelin is unsatisfied to the extent of about one quarter, or upon two atoms of nitrogen there is very nearly one and a half molecle of cadmium chloride (1·48).

Synopsis of the foregoing Analytical Results.

	Percents.	÷At. Wgts.
C	50·70	
H	9·08	
N	2·28	0·162
P	3·24	0·104
O	11·28	
Cd, Cl_2	23·42	
	100·00	

Calculation of Elements in 100 *of Organic Molecle.*

	Percents.	÷At. Wgts.	÷N = 2.
C	66·21	5·5175	51·8
H	11·86	11·860	111·2
N	2·98	0·213	2·
P	4·23	0·136	1·26
O	14·73	0·921	8·64

The relations of C : N are the same as those of the same elements in the crystallised free body, which will be described further on. Consequently the phosphorus is somewhat too high.

Further Purification of the Compound by Ether, and by recrystallisation.—The mixture was now subjected to a process of purification by being exhausted in a closed apparatus with boiling ether. When new ether failed to extract anything during an entire day of boiling, the compounds were recrystallised from spirit, again dried, and again exhausted with ether. If now fresh ether extracted nothing, the salts were considered to be fully exhausted. (Some krinosin and bregenin were extracted.) In this manner a number of preparations were purified, with the result that in all the carbon sank a little, while cadmium chloride rose in quantity, but nitrogen remained to phosphorus as 2 : 1·28, as will be seen from the following analyses.

Synopsis of Quantations and Theory of Salt.

	Percents.		÷At. Wgts.
C	46·11		3·84
H	8·66		8·66
N	2·24		0·160
P	3·22		0·104
O	14·37		
Cd	15·63	25·40	
Cl	9·77		

Elements and Theory of Organic Matter.

	Percents.	÷At. Wgts.	÷N = 2
C	61·80	5·15	48·5
H	11·60	11·60	
N	3·00	0·214	2·
P	4·31	0·139	1·28
O	19·29	1·205	

We have therefore here also the phosphorus too high for sphingomyelin, namely, N : P = 3 : 2, from which it may be surmised that the admixture was partially a mononitrogenised phosphatide (paramyelin ?), partly a diphosphatide such as will be described further on.

As no even relation between nitrogen and phosphorus could be obtained by any kind of treatment, particularly frequent recrystallisation, to which the compound was subjected, it was deemed necessary to decompose it and study the free body. It was evident from the relation C : N = 51·8 : 2 that the organic body was not a phosphatide of the lecithin group, in which C : N = 42 : 1,

or thereabouts, while the amount of nitrogen pointed to the probability that the body was constituted similarly to the amidomyelins which had been hypothetically assumed to exist in analysed $CdCl_2$ and $PtCl_4$ compounds. In fact, it was at once perceived to present the proportions between C and N which had been observed upon *apomyelin* described in my first research on the brain. But as it had been left doubtful whether this apomyelin was not a product rather than an educt, great care was taken to prevent any chemolytic influence from acting on the body, so that the doubt just mentioned might be eliminated. The crystallisation of the free substance showed at once the presence of *two principles*, of which the first, *sphingomyelin*, was obtained pure by crystallisation, while the second one was obtained pure by more circumstantial processes to be described later.

Preparation of pure Sphingomyelin from its Cadmium Chloride Compound.

Removal of the Cadmium by Diffusion and Dialysis.—When the compound is placed in water, this will extract much of the $CdCl_2$, but not all, by dissociation and diffusion. But the liberated sphingomyelin is in the state of colloidation, which increases with the length of contact with water until it reaches its maximum. The liquid can thus no longer be filtered from the sphingomyelin, as the latter immediately obstructs the pores of the filter.

Dialysis may be resorted to, the apparatus being arranged with plaited vegetable parchment dialysers as described on p. 101. When the dialysate is free from $CdCl_2$, the sphingomyelin and water paste must be concentrated, dissolved in hot spirit, treated with hydrothion to remove the last portions of cadmium, and set to crystallise.

The sphingomyelin which was examined in the first four analyses to be related below was prepared with the aid of dialysis, as follows: 40 g. $CdCl_2$ salt was boiled with 1 litre spirit; 23 g. dissolved, while 13 g. remained insoluble in the quantity of spirit mentioned. The hot spirit solution deposited a quantity of salt above 30°, and was filtered at 30°. The salt thus obtained was subjected to dialysis, and washed with water until free from chlorine. It was then recrystallised from spirit, and dried at 100°.

The liquid which had deposited the $CdCl_2$ salt above 30° was

evaporated, and the deposit filtered off. It was also decomposed with water, and the body free from $CdCl_2$ was analysed. It yielded results which have been recorded separately. If my object were solely that of a mere chemist in search of new pure compounds, I should have thrown this and other mother-liquors away. But the object of physiological and pathological research is not to find some principles which will pay the chemical operator, but *to find all the principles* which may be contained in an organic physiological or pathological mixture.

The following process yields sphingomyelin in a shorter time than dialysis. The purified salt, free from kerasin and all matters which ether can extract, is suspended in spirit, and treated with hydrothion to saturation. The flask containing the mixture is now placed in a water-bath, the water of which is gradually raised in temperature until the spirit in the flask boils. The influence of the sulphuretted gas is continued until a filtered sample of the spirit solution is not changed by the hydrothion any more. The mixture is now filtered through paper in a funnel kept hot by a steam-jacket. The filtrate is left to crystallise. It yields sphingomyelin, etc., partly as hydrochlorate, which are collected on a filter, washed with spirit, pressed between bibulous paper, and dried in vacuo over oil of vitriol. The neutral and dry salts are now again dissolved in warm spirit, and to the solution is added gradually, and in small quantities, as much finely pulverised mercuramin as may be necessary to bind and retain in the precipitate all the hydrochloric acid combined with the phosphatides. When an excess of mercuramin is present—*i.e.*, when the deep canary-yellow colour of the mercuramin is no longer changed to white, but remains as a yellow deposit at the bottom of the flask, distinct from the white mercuramin hydrochlorate—then the organic principles are free from hydrochloric acid; the filtered solution, on cooling, deposits first sphingomyelin, to be purified by recrystallisation; the concentrated mother-liquor deposits the mononitrogenised companion mainly. (Without the mercuramin treatment the sphingomyelin may retain from 0·8 to 1·32 per cent. HCl.)

Physical and Chemical Properties of Sphingomyelin.—Sphingomyelin is easily soluble in hot spirit or absolute alcohol, and crystallises therefrom in dense white masses, needles, stars, and hexagonal plates. It does not become waxy after drying, but

retains a pulverulent dryness. It is little soluble in cold absolute alcohol, but can be separated thereby from phrenosin, which is less soluble, and from lecithin, which is more soluble. It is almost insoluble in ether, even when some hydrochloric acid has been added. It cannot be separated from kerasin, to which it stands in the relation of a base, by recrystallisation alone. The intervention of cadmium chloride is necessary to effect this separation. The compound of sphingomyelin with cadmium chloride is less soluble in spirit than the pure sphingomyelin, and is deposited in a much shorter time and at higher temperatures than kerasin; for the sphingomyelin $CdCl_2$ falls above 28°, kerasin (in solutions which contain less than 1 g. in 321 cc. of spirit of 84 per cent.) below 28°, on standing. Sphingomyelin swells in water, and becomes distributed in it so as to form a turbid semisolution or emulsion. In the course of dialysis it sometimes contracts and sinks in the water. Its compounds, particularly those with $CdCl_2$, become decomposed under the influence of water; sphingomyelin assumes the colloid form, and the combined metals or salts pass into solution in the water. On this property rests the process for liberating sphingomyelin from crystalloids by dialysis.

Quantations of Elements in Sphingomyelin from Ox Brain. Synopsis of Analyses and Theory.

	Percents.	÷At. Wgts.	÷P=1.
C	65·37	5·445	51·86
H	11·29	11·29	107·52
N	2·96	0·211	2·
P	3·24	0·105	1·
O	17·14	1·070	10·2

The probable formula is $C_{52}H_{104}N_2PO_9 + H_2O$.

Comparison of Sphingomyelin with Apomyelin from Human Brain.

In 'Reports,' No. III. (1874), p. 164, I have given the analysis of a specimen of a kind of myelin from the human brain which I termed *Apomyelin*, and which had been obtained from a platinic chloride compound by recrystallisation from boiling alcohol, and decomposition of the product by hydrothion. The following figures were given:

Apomyelin, Man.

	Percents.	÷At. Wgts.	÷P = 1.
C	67·01	5·5841	54
H	11·35	11·35	109
N	3·00	·2142	2
P	3·23	·1041	1
O	14·694	·9155	9
Cl	0·761		

This was, therefore, clearly a sphingomyelin with 54 C; like the sphingomyelin from the $CdCl_2$ compound, it retained a small quantity of hydrochloric acid, which was estimated in the analyses and recorded as Cl. A sphingomyelin prepared from $CdCl_2$ salt by H_2S, was found to retain 1·32 per cent. HCl, which is only about a third of the theoretical amount required by a simple hydrochlorate. In both cases the hydrochlorate was probably decomposed by the influence of watery solvents.

Chemolyses of Sphingomyelin, with a view of ascertaining its Chemical Constitution.

I have made four distinct experiments on this subject; but as they are not completed, I am only able to state the salient results of the operations as far as they go.

Experiment I.—Six g. sphingomyelin were mixed with 12 g. barita hydrate and heated to 105° during five hours. There were obtained a little *sphingosin* (precipitated from the alcoholic extract by sulphuric acid, soluble in excess), an acid which differed from sphingomyelin by the absence of the group of *neurin*, *sphingomyelic* acid, probably (if the sphingomyelin employed had the formula $C_{53}H_{106}N_2PO_{12}$) having the composition $C_{48}H_{95}NPO_{12}$. In this acid, it will be observed, P : N = 1 : 1. There was further obtained a *barium salt soluble in ether*, and an alcohol *sphingol*, the two latter bodies being products of the continued decomposition of a portion of sphingomyelic acid.

Experiment II.—Twelve g. sphingomyelin, 12 g. barita and 50 g. water were heated to 100° for ten hours. There were obtained 1·1 g. of neurin as platinic chloride salt, while theory requires 1·3 g. from 12 g. sphingomyelin. *No trace of glycerophosphoric acid was observed.* There was a barium salt insoluble in alcohol and ether, which contained P : N = 1 : 1·02 (P = 2·75 per cent.; N = 1·27 per cent.) sphingomyelate of barium. No sphingosin

was obtained. But the new alcohol, *sphingol*, was isolated in sufficient quantity to be analysed.

Analysis of Sphingol, a new Alcohol, from Sphingomyelin by Chemolysis with Barita.

Dried at 98° in a platinum boat.

Synopsis of Analyses and Theory.

		Percents.	÷At Wgts.	÷by O = 1.
(1)	C	76·30	6·37	9·07
	H	12·78	12·78	18·7
	O	10·92	0·68	1·
(2)	C	76·34	6·361	9·08
	H	12·45	12·45	17·92
	O	11·21	0·7	1·

The new alcohol has therefore the composition expressed by either $C_9H_{18}O$, or by $C_{18}H_{36}O_2$. In the latter case it would be the third stearic isomer.

Analysis of the Neurin-Platinum-Chloride Hydrochlorate obtained from this Chemolysis.

The salt $C_{10}H_{26}N_2O_2$, $2HCl,PtCl_4$, of atomic weight 618·4,

	Requires in 100.	Found in Salt.
Pt	31·921	31·79
Cl	34·44	34·64

Experiment III.—Twenty g. sphingomyelin and 40 g. barita, with 500 g. of water, were heated to 135° during twelve hours. There were obtained *neurin*; further a second *alkaloid* precipitated by sulphuric acid from its solution in absolute alcohol, yielding as sulphate by analysis the formula $2(C_{20}H_{41}NO_2)H_2SO_4$, being possibly somewhat impure sphingosin; sphingol; a fatty acid, which as barium salt and as lead salt was insoluble in alcohol and in ether, in the free state had the composition expressed by the formula $C_{18}H_{36}O_2$, crystallised, and fused at 57°, therefore almost as much below ordinary stearic acid, which fuses at 69·5°, as neurostearic acid fuses above this, namely, at 84°. The acid is therefore the fourth isomer of stearic acid, the third discovered in these researches, and is named *sphingostearic acid.*

No glycerophosphoric acid was obtained, but the insoluble barium salt contained much *barium phosphate*, which was isolated;

the phosphoric acid was combined with molybdate of ammonium, and fully identified.

Experiment IV.—Twenty-five g. sphingomyelin, 50 g. barita, and some water were heated in the autoclave to 105°—120° for seventeen hours. There were again obtained neurin, sphingosin, sphingol, barium salt insoluble in ether, and a neutral body mixed with sphingol; this latter mixture or compound had the formula $C_{35}H_{81}NO_5$, with 2·18 N.

Some intermediate, in quantity subordinate, products, such as a barium salt soluble in ether and precipitable by alcohol, remain for further study.

Theoretical Results of these Chemolyses.

The conclusions to be derived from these chemolyses bearing upon the chemical constitution of sphingomyelin are of the utmost interest and importance.

In the first place, no glycerophosphoric acid was obtained in any one of the chemolyses. If I recall the many experiments made regarding the constitution of other phosphatides, such as of kephalin or of lecithin, and the relative facility with which glycerophosphoric acid was isolated as barium salt and identified as calcium salt, I cannot suppose for a moment that glycerophosphoric acid has been present and escaped observation. I therefore came to the conclusion that the glycerol found in a portion of the phosphatides was not, as has been hitherto supposed, essential to the composition of those bodies. From this moment it was also evident that glycerol could not, or need not, be the basal radicle of these compounds, not even of those which contained it, and that therefore the phosphorised brain educts could no longer be considered as glycerides, or ethers of the alcohol glycerol, but had to be differently interpreted. The only feature common and essential to all the phosphorised educts, and the one from which in consequence they derived their appellation, was that they contained phosphorus, which by ordinary analytical processes remained in the form of phosphoric acid. There was no reason to suppose that the phosphorus was present in the organic principles in any other form than that of phosphoryl, the radicle of phosphoric acid. (When they were chemolysed they took up water, and yielded hydrated radicles, such as have been described, acids, alkaloids, or organic bases, and alcohols.) Consequently I

assumed that *the basal radicle of all phosphatides was that of phosphoric acid; that in this acid one, two, or three molecles of hydroxyl might be replaced by radicles of alcohols, acids, or bases, and that to a molecle formed by three such substitutions there might yet be attached, by substitution of an element in a radicle, itself already substituted* (side-chain) *or by addition as anhydride, a fourth radicle,* and that therefore sphingomyelin might be constituted according to the following formulæ:

$$\text{Phosphoric acid} = OP \begin{cases} HO \\ HO \\ HO \end{cases}$$

$$\text{Sphingomyelin} = OP \begin{cases} \text{Acid radicle.} \\ \text{Alcohol radicle.} \\ \text{Base radicle (substituted).} \\ \text{Base radicle (attached as side-chain or} \\ \quad \text{added as anhydride).} \end{cases}$$

I repeat the formula, substituting elementary formulæ for the functional symbols:

$$OP \left\{ \begin{matrix} C_{18}H_{35}O_2 \\ C_{18}H_{35}O_2 \\ C_{17}H_{34}O_2N \\ C_5H_{11}N \end{matrix} \right\} = C_{58}H_{115}N_2PO_7.$$

To this formula we are obliged, by the results of the analyses of the three sphingomyelins, to add at least $2H_2O$, perhaps $3H_2O$, and by this operation we obtain a total formula of $C_{58}H_{121}N_2PO_{10}$. But this last formula is only a case out of many possible formulæ. It is clear that if the molecular formula of sphingol were only $C_9H_{18}O$, the formula above given would either have to be lowered to C_{49}, etc., or the sphingol would have to be assumed to be doubly represented. Neither hypothesis enjoys the advantage of probability. I therefore do not presume to fix the exact formula of sphingomyelin, particularly as I have shown that there are various sphingomyelins (one being apomyelin), but I maintain that this research has established as a type of brain-educts sphingomyelin, which is a phosphatide, and contains an alcohol not being glycerol, an acid, and two nitrogenised radicles as proximate constituents.

Compounds of Sphingomyelin.—The body combines with *hydrochloric acid,* and this compound is more soluble in spirit than the free body. The hydrochlorate is easily decomposed by watery solvents, so that while a monohydrochlorate should contain 3·76

per cent. HCl, there were found in a hydrochlorate recrystallised from spirit only 1·32 per cent. HCl.

Compounds of Sphingomyelin with Cadmium Chloride.—The varying quantities of cadmium chloride in such compounds have been a source of great trouble and doubt, which could only be solved by countless preparations and analyses piloted by hypotheses and the atomic theory. Thus a salt was found to contain 16·86 per cent. $CdCl_2$, and to correspond to the formula $C_{51}H_{99}N_2PO_{10}CdCl_2$, which requires 16·4 per cent. $CdCl_2$.

Another salt from the human brain contained 26·59 per cent. $CdCl_2$, and was therefore supposed to be a not quite saturated compound approaching that with two molecles of $CdCl_2$. For the C_{51} sphingomyelin with $2CdCl_2$ requires 28 per cent. $CdCl_2$; the formula with C_{52} requires 27·9 per cent. $CdCl_2$.

Sphingomyelin is therefore, like amidomyelin, a dipolar alkaloid, and capable of fixing one molecle of $CdCl_2$ to each of its two nitrogen radicles.

Consequently any sphingomyelin and $CdCl_2$ compound which contains quantities of $CdCl_2$ intermediate between 16·4 per cent. and 28 per cent. is a mixture of the monocadmium chloride, with the dicadmium chloride compound. Such a compound must of course contain $P : N = 1 : 2$. Where these relations do not subsist, but where the relations of $P : N$ approach more or less those of $2 : 3$, the cadmium chloride may be diminished by the presence of a compound with it of a phosphatide such as paramyelin, in which $P : N = 1 : 1$. It will therefore be seen that the higher the amount of $CdCl_2$ found in a new precipitate, the more likely is the body to contain sphingomyelin (or amidomyelin). If the $CdCl_2$ sinks much towards 20 per cent. or below, the precipitate or crystallisation either contains much of the monocadmium chloride compound, or contains a mononitrogenised body, which may be either a body analogous to sphingomyelin, but containing one nitrogenised radicle only, or a lower phosphatide such as lecithin or paramyelin.

We have therefore, with regard to sphingomyelin, the possibility of the existence of two compounds and an indefinite number of their mixtures in different proportions

$C_{51}H_{99}N_2PO_{10} + CdCl_2$, At. W. = 1113, contains 16·4 per cent. $CdCl_2$.

$C_{51}H_{99}N_2PO_{10} + 2(CdCl_2)$, At W. = 1296, contains 28 per cent. $CdCl_2$.

If there are, as is probable, many sphingomyelins, then each variety would have its several $CdCl_2$ compounds.

All these $CdCl_2$ salts are beautifully crystallised and white. When they are recrystallised from spirit they lose solubility as the extraction proceeds. These changes are particularly observed upon salts which are not fully saturated with $CdCl_2$: the fully saturated salts behave in a regular and stable manner. It is therefore necessary in the first crystallisation to offer to sphingomyelin an excess of cadmium chloride, and to test the mother-liquors of recrystallisation from time to time to see that they do not contain unsaturated more soluble salt or free sphingomyelin in solution. Spirit of 85 per cent. strength, after having been saturated boiling with the dicadmium chloride salt and allowed to deposit all it can during twenty-four hours, will keep in solution half a gramme of the salt in 100 cubic centimetres.

C. SUBGROUP OF DINITROGENISED DIPHOSPHATIDES. N : P = 2 : 2.

ASSURIN : ITS ISOLATION AND ANALYSIS.

This body is found in the alcohol extracts of the cerebrin mixtures after sphingomyelin and kerasin have been removed from them in the manner above indicated. When to such a solution platinum chloride, acidified with some hydrochloric acid, is added, a precipitate ensues which is insoluble on boiling. In solution there remains a body, which I have termed *Istarin,* and which does apparently not combine with platinum chloride. Both bodies, assurin and istarin, seem to have some attraction for each other, like sphingomyelin and kerasin, which causes them to crystallise together, so as to represent a uniform appearance of star-shaped masses of crystals. This union is apparently never definite, but the proportions of the ingredients shift according to the mass, concentration, and temperature of the solvents used for their extraction. On the whole, frequent recrystallisation from spirit causes the phosphorus in the mixture to rise, and the nitrogen to sink relatively to the phosphorus. But the principle here to be described is isolated as yet only by platinum chloride.

Assurin Hydrochlorate Platinum Chloride.

Yellow crystalline powder, insoluble in boiling spirit, and in ether.

Synopsis of the Results of the First Series of Analyses and Theory.

	Percents.	÷ At. Wgts.	÷ Pt = 1.	Organ. Molecle.
C	49·25	4·104	91·20	45·6
H	8·74	8·740	194·22	97·0
N	2·50	0·178	3·95	1·97
P	6·52	0·210	4·66	2·33
O	13·69	0·855	19·00	9·5
Pt	9·03	0·045	1·00	
Cl	10·27	0·289	6·42	

These data lead to a formula $2(C_{46}H_{94}N_2P_2O_9HCl)PtCl_4$.

Synopsis of the Results of the Second Series of Analyses and Theory.

	Percents.	÷ At. Wgts.	÷ N = 2.	÷ Pt = 1.	Organ. Molecle.
C	49·01	4·084	49·8	94·3	47·
H	8·85	8·85		203·	101·
N	2·30	0·169	2·	3·77	1·88
P	6·06	0·1954	2·2	4·49	2·24
O	15·66	0·98		22·	11·
Pt	8·57	0·0435		1·	
Cl	9·55	0 269		6·18	

These data lead to a formula $2(C_{47}H_{101}N_2P_2O_{11}HCl)PtCl_4$, which differs a little from the former one, but phosphorus remains slightly exceeding, nitrogen below the theory derived from the platinum chloride as starting base. Assuming nitrogen at two atoms, we come to nearly 50 C, but encounter again an excess of 10 per cent. in the phosphorus. But the great features of the results of the quantations are evident. We have to deal with a phosphatide in which the radicle of phosphoric acid is contained twice, and which we may therefore term a *diphosphatide.* In this principle there are contained two atoms of nitrogen, which, from analogy with other phosphorised bodies, we may suppose to be contained in two different nitrogenised radicles. But even if the two atoms of nitrogen were contained in one and the same radicle, it would still be perfectly correct to term the principle a dinitrogenised diphosphatide. For the nitrogen, although somewhat deficient in both sets of analyses, amounts nevertheless to 3·77 molecles in the latter and to 3·95 in the first set of quantations when compared to platinum as 1.

The companion of assurin, the above-mentioned *istarin*, is not phosphorised, but it is very difficult to prepare it free from the last traces of phosphorus. Its chemical composition is expressed

approximately by the formula $C_{40}H_{82}NO_6$; it therefore seems to belong to the group of nitrogenised fats to be described below. I have prepared and analysed many specimens which have shown the way to the ultimate complete chemical individualisation of the substance. But there has not been time for the carrying out of the laborious operations which are necessary for the isolation of the quantities required. For it must be borne in mind that before istarin is reached in a systematic course of brain analysis all the other substances described previously must have been removed out of the solution, as well as the residues of the solvents and precipitants.

D. SUBGROUP OF NITROGENISED PHOSPHATIDE-SULPHATIDES.

Body from Group of Cerebrinacides.

Such a principle I shall have to describe under the subgroup of the cerebrinacides, amongst which it occurs, and from which it has not yet been entirely isolated. Indeed, it may be questioned whether this cerebrosulphatide contains phosphorus as a constituent element, or only as a constituent element of an admixture. The observation, such as it is, is too important to be left out of sight; and, on the other hand, nothing but a research of great dimensions will be the means of evolving the final truth contained in it.

E. SUBGROUP OF NONNITROGENISED MONOPHOSPHATIDES.

A body typical of this subgroup was discovered by limited chemolysis of kephalin, as described in the chapter relating thereto, under kephalophosphoric acid. Two other bodies of this kind were found in a lead precipitate from buttery matter. One was a crystallised acid, the other noncrystallised. There has not been time for advancing the knowledge concerning them; particularly the proof is yet wanting that they are educts, and not, like kephalophosphoric acid, products. They are, therefore, registered here mainly as objects for future research.

First acid from buttery matter.
Second acid from buttery matter.
Kephalophosphoric acid (product).

From the mixture of the first two acids a barium salt soluble in ether was obtained, reminding of the bearing of the kephalin and sphingomyelin series, which alone, as thus far known, yield barium salts soluble in ether.

F. COMPARATIVE CONSIDERATION OF OTHER PHOSPHATIDES OF THE ANIMAL BODY.

Phosphatide of the Milk, Lactophosphatide, Casein.

According to the latest researches, casein from cows' milk has the following percentic composition :

		÷ At. Wgts.	Atoms.
C	52·96	4·413	197·8
H	7·05	7·05	316·
N	15·65	1·1178	50·
S	0·716	0·0223	1·
P	0 847	0·0273	1·224
O	22·78	1·4237	63·8

It will be seen that what has often been maintained before is here again propounded—namely, that a substance believed to be truly albuminous contains not only sulphur, but also phosphorus, as an essential constituent. On general grounds I think this very probable. Indeed, the phosphatides of the brain have some properties which are so much like those of casein that former inquirers were led by them to the belief that the brain did actually contain casein. As casein yields by chemolysis about 4·12 per cent. of tyrosin, and as the atomic weight of the latter is 181, the atomic weight of casein must be at least 4393. Now if casein contained one atom of sulphur, its atomic weight would thereby be fixed at about 4469, which does not differ much from the number derived from the tyrosin ; but the phosphorus leads to a lower number—namely, 3659. Seeing, however, that phosphorus is analytically always found a little too high, we need not at first sight attribute too much importance to this difference. Seeing, on the other hand, that albumen contains at least three atoms of sulphur, we need not despair of finding, by further inquiry, a better ratio between the phosphorus and sulphur in casein than that which is at present apparent.

Phosphatides of the Bile, Cholophosphatides.

It is generally assumed that the bile contains lecithin. This assumption is based upon the fact that ox-bile, by chemolysis with barita, yields fatty acids and neurin. Indeed, neurin was first discovered in the bile, and originally termed cholin. I have made some experiments regarding this question, and come to the result that ox-bile does not contain lecithin, but contains a phosphatide which, to conclude from its crystallising as platinum chloride salt, seems to have a very complicated composition. The formula expressing the composition of the platinum chloride compound with P = 1 was $C_{82}H_{164}N_4PO_{36},HCl + 2PtCl_4$. The fact that bile, a secretion which serves the chemistry of digestion and assimilation, contains, besides its specific ingredients, bodies which, like cholesterin, are identical with important ingredients of the brain, or, like this cholophosphatide, are analogous to them, shows that the biolytic or biosynthetic process which leads to the formation of bile is much more complicated than has hitherto been supposed. One of the principal fatty acids in the bovine cholophosphatide is stearic.

Phosphatides of the Blood, Hematophosphatides.

A phosphatide was found mixed with a preparation of hemine crystals made according to Rollet's prescription. It was extracted by benzol and acetic acid, and from the residue of this solution by hot absolute alcohol. From this solution it was precipitated as $CdCl_2$ salt; the white salt was recrystallised and analysed. It led to an empirical formula, $C_{76}H_{164}N_3P_2O_{14} + 2CdCl_2$. From this it is probable that the salt was a mixture of a mononitrogenised with a dinitrogenised phosphatide—if a conclusion may be drawn from its physical appearance, paramyelin and amidomyelin. It had almost the same percentic composition as a similar preparation of a $CdCl_2$ salt obtained from brain. We may assume that this phosphatide came from the blood-corpuscles, like the hematin which it accompanied, and was an element of their bioplastic constitution. Whether it was in any way centralised, like the phosphatides of cell-nuclei, cannot be stated.

From an ether extract of blood-corpuscles of the ox I obtained a cadmium chloride precipitate, of which a portion was soluble in cold benzol—lecithin cadmium chloride; while another portion was insoluble in boiling benzol—amidomyelin cadmium chloride.

Phosphatides of Nucleolar Centres of Growth (Bioplasm, Cells, etc.), Cytophosphatides.

Aggregations of cells, whether vegetable, such as yeast, or animal, such as sperma, pus, or liver (the latter after removal of bloodvessels and connective-tissue), are known to contain a peculiar phosphorised substance. As this substance was supposed to be contained in or to constitute the nucleus, it was called nuclein. This term would be unobjectionable if all tissue elements yielding the substance contained nuclei. However, for our present purpose we will consider mainly those which do contain nuclei, and leave the others for future consideration.

The nucleolar matter has mostly been obtained by subjecting the cells to a process of artificial digestion. The undigested (*i.e.*, undissolved) part was supposed to be unaltered nucleolar matter. This was now extracted with dilute caustic alkali, and from the filtered solution the nuclein so-called was precipitated by dilute hydrochloric acid. This was washed with alcohol and ether, dried in vacuo, and analysed. Other authors avoided the process of digestion, and extracted the mass of cells, such as German yeast, with soda lye directly, and treated the filtrate as just described. Nuclein from yeast thus obtained gave from 40·42 to 41·22 per cent. of C, 5·15 to 5·52 per cent. H, 15·31 to 15·99 per cent. N, 6·1 to 6·29 per cent. P, and 0·38 to 0·41 per cent. S; or—

	Means.
C	40·81
H	5·38
N	15·76
P	6·19
S	0·39

But a great number of other analyses gave only from 2·58 to 3·98 per cent. P. The nuclein from pus-corpuscles gave from 2·28 to 2·62 per cent. P, about 1·7 per cent. S, and from 14 to 15·02 per cent. N, besides 49·58 per cent. C, and 7·10 per cent. H. Nuclein from the red blood-corpuscles from geese gave 6·04 to 7·12 per cent. P, and 0·4 per cent. S; while so-called nuclein from the so-called nucleolar formations of the yelk gave 7·10 per cent. P, 0·99 per cent. S, and 13·46 per cent. N.

From this it will be evident that the science of the nucleins is only in course of development, and that there are at present

two groups of these substances known—one with from 6 to 7 per cent. P, another with from 2 to 3 per cent. P. The latter is the better known. It yields, by long boiling with water, *phosphoric acid*, an *albuminous substance* soluble in water, and a *peptone*, and a mixture of the three alkaloids, *hypoxanthin*, *xanthin*, and *guanin*. From the study of the phosphatides of the brain, we can have no difficulty in explaining such a body hypothetically to be a phosphatide. The body with the high amount of phosphorus we could comprehend to be a di- or triphosphatide; whereas the body with the lesser amount of P would have a more simple structure. However, our only object in this place is to direct attention to these bodies also; for there can be no doubt that the knowledge of each set of organic principles will enable us to advance that of the others.

G. INORGANIC BASES EXISTING IN THE BRAIN IN COMBINATION WITH PHOSPHATIDES.

The phosphatides, as they exist in brain-matter, and as isolated therefrom, are associated with certain bases, by a power of combination derived from their acid character. These bases can only be removed and the principles obtained in a pure state by dissolving the compounds in water, and adding hydrochloric acid, which combines loosely with the principles on account of their alkaloidal character, to the exclusion of the bases, which then pass into solution as chlorides.

In order to obtain some knowledge regarding the relative amounts and nature of the bases in combination with the phosphorised constituents of brain-matter, a quantity of solution obtained by the hydrochloric acid process, above described, and which was derived from kephalin, paramyelin, and lecithin, was submitted to analysis.

It was evaporated to dryness, and the residue ignited in a previously weighed platinum dish. During this ignition much chloride of ammonium was given out, and its nature distinctly proved by condensing and analysing it.

The fused mass was dissolved in dilute hydrochloric acid, and the solution so obtained treated with excess of ammonia. The white gelatinous precipitate which was thrown down was scarcely coloured black by sulphide of ammonium. The precipitate and filtrate were submitted to qualitative and quantitative analysis.

The Precipitate.—The colouring-matter was evidently sulphide of iron, but it did not amount to more than a trace. [The *original* solution had been subjected in a neutral state to a current of sulphuretted hydrogen with the object of removing some platinum that had been used along with the hydrochloric acid, as $PtCl_4$, in the precipitation of the kephalin and myelin from the aqueous solutions. This treatment must, therefore, have removed any copper and iron which were undoubtedly present.]

The precipitate was dissolved in dilute hydrochloric acid, and the calcium, magnesium, and phosphoric acid estimated as follows:

The solution was nearly neutralised by sodic carbonate, after addition of some ferric chloride, and then an excess of pure baritic carbonate was added; the mixture was shaken, allowed to stand, and then filtered.

From the concentrated filtrate the barium was removed by sulphuric acid, and the filtrate was then precipitated in the presence of ammonia by oxalate of ammonium. The oxalate so obtained was converted by intense ignition into oxide, CaO, which weighed 0·1994 g. = 0·1423 g. calcium.

The filtrate and washings from the calcium oxalate were treated as ordinarily in cases where it is desired to estimate magnesium, and there was obtained 0·5555 g. $Mg_2P_2O_7$ = 0·1212 g. magnesium.

The precipitate which had been produced by ferric chloride and sodium and barium carbonates was dissolved in nitric acid, and the phosphorus estimated by the combined molybdate and magnesium methods. This gave 0·3266 g. phosphorus = 0·7480 g. phosphoric acid (P_2O_5).

The Ammoniacal Filtrate.—This was proved to be free from magnesium and calcium. The potassium contained in it was estimated in the usual way by means of platinic chloride, giving 11·2766 g. of $2KCl,PtCl_4$ salt = 3·651 g. KCl = 1·911 g. potassium. The mother-liquor and washings were evaporated to dryness, and the platinum was removed by extracting the ignited mass with water acidified by hydrochloric acid. The extract so obtained was evaporated to dryness and fused; the residue weighed 3·45 g. (NaCl) = 1·356 g. sodium. The quantities of potassium and sodium thus found were controlled by the analysis of a separate part of the original solution containing their chlorides, and cal-

culating from the residue obtained on evaporation, there should have been found :

A total quantity of chlorides	= 7·09	g.
There was found -	KCl = 3·651	„
	and NaCl = 3·450	„
	or a total of = 7·101	„

Distribution of the Bases and Phosphoric Acid.—Assuming the calcium and magnesium to have originally existed as tribasic phosphates, and any phosphoric acid remaining over to have been in combination with potassium, then we have :

Ca = 0·1423 g.	existing as	$3CaO,P_2O_5$.
Mg = 0·1212 „	„	$3MgO,P_2O_5$.
K = 0·5663 „	„	$3K_2O,P_2O_5$.
K = 1·3447 „	„	} combined directly with kephalin,
Na = 1·356 „	„	} paramyelin, and lecithin.
P = 0·3266 „	„	P_2O_5 (0·748 g.) and distributed between the Ca, Mg, and K.

It has been proved above that kephalin was partly in combination with calcium oxide, or lime, uncombined with any other acid. In the present research we find that there may be in kephalin (and myelin) even a much greater quantity of potash and soda in direct combination than of lime. Both observations supplement each other, the first one being qualitative only, the present one stating the quantities of the bases relatively to each other.

H. SPECIAL STUDY OF GLYCEROPHOSPHORIC ACID AND ITS SALTS, AS OBTAINED FROM SOME PHOSPHATIDES OF THE BRAIN.

Glycerophosphate of lead may be prepared from the solution of barium glycerophosphate as obtained by the chemolysis of phosphorised matters, by precipitation with any soluble salt of lead, as, for example, the chloride or acetate. In order to entirely remove glycerophosphoric acid in this way from the solution, it is necessary to concentrate and neutralise the latter from time to time, on account of a slight solubility of the glycerophosphate of lead.

Glycerophosphate of lead prepared synthetically is granular and white, and remains so on drying, whereas when it is obtained

from kephalin it dries to a hard, brittle, slightly coloured mass, even when chemically pure.

On ignition, the salt leaves a residue of pyrophosphate of lead, and this offers a ready means of ascertaining the purity of the preparation. Thus, with a specimen of the salt prepared as described from kephalin, it was found that 0·911 g. left a residue of 0·744 g. $Pb_2P_2O_7$, while theory requires 0·7104 g.

Glycerophosphate of calcium (normal salt) may be prepared from the lead salt by decomposition with hydrothion, and neutralisation of the filtrate with calcium carbonate.

This salt is much less soluble in a hot concentrated aqueous solution than in the same amount of water in the cold. It is therefore best isolated by filtration of a precipitate from the nearly boiling solution.

A quantity of the lead salt, referred to above, was converted into calcium salt, and the solution evaporated near the boiling-point, when a white deposit of calcium glycerophosphate formed. This, when washed and dried, was analysed. 0·3400 g., after strong ignition with the aid of nitric acid, left a residue of 0·207 g., equal to 60·8 per cent. $Ca_2P_2O_7$. Pure calcium glycerophosphate should leave 60·5 per cent. pyrophosphate.

Another sample of the calcium salt prepared as described gave the following figures on analysis :

		Percents.	Atoms.
C	=	16·30	2·9
H	=	3·47	7·3
Ca	=	18·96	1·0
P	=	14·69	1·0
O	=	46·58	6·1

which shows the composition of this salt to be according to the formula $C_3H_7CaPO_6$.

Acid Glycerophosphate of Calcium.—A solution of the calcium salt neutral to test-paper becomes on heating, and at the same time as the normal salt separates, acid to test-paper; and if the mother-liquor is now precipitated with alcohol, there is produced, not the normal but the acid salt. A portion precipitated in this way by alcohol was white and granular. It was dried at 100° C.

0·827 g. left on ignition 0·478 g. residue = 57·80 per cent. Now the normal salt would have given, as we have seen, 60·5 per

cent. residue. This observation led to the theory of an acid salt of this construction, $C_3H_7CaPO_6,C_3H_9PO_6$, giving a total formula of $C_6H_{16}CaP_2O_{12}$; and it was probable that on ignition such a salt would leave a residue of half-saturated acid pyrophosphate $H_2CaP_2O_7$, losing only $C_6H_{14}O_5$.

On this theory, the residue should have amounted to 56·54 per cent.; it did amount to 57·80 per cent.

Glycerophosphate of barium prepared from synthetically made acid and barium carbonate was white, and behaved, as regards its insolubility in, and consequent precipitation from hot aqueous solution, similarly to the calcium salt. But this property of separation of the salt on boiling the solution seemed to be only transient, for after a solution had stood for sixteen hours after it had been so precipitated, no precipitation occurred on again boiling the solution, and ammonia produced a voluminous precipitate in the solution, apparently indicating that a partial decomposition of the salt had occurred.

Some of the salt was prepared from the solution resulting from the decomposition of kephalin by barita, by precipitation with alcohol. This was redissolved in water, and reprecipitated by alcohol several times; finally it was dissolved in water, and concentrated by evaporation on the water-bath, when a deposit occurred. This was isolated, dried by pressure between folds of paper, and over H_2SO_4 in vacuo, and finally at 100° C. At 110° C. it was not affected; it was now analysed.

		Percents.	Atoms.
C	=	11·46	2·9
H	=	2·44	7·4
Ba	=	45·15	1·0
P	=	10·21	1·0
O	=	30·74	5·8

leading to formula $C_3H_7BaPO_6$.

Another specimen of barium glycerophosphate was prepared much in the same way, but with this difference, that whereas the one, the analysis of which has just been given, was separated from water by boiling, the one now to be described was precipitated from aqueous solution by alcohol, with which it was also washed. On isolation the precipitate contracted, became horny, transparent, and finally fused to a thick liquid. It eventually dried to a brittle mass. On analysis it gave :

		Percents.
C	=	12·611
H	=	2·933
Ba	=	40·950
P	=	9·266
O	=	34·240

leading to formula $C_3H_7BaPO_6,H_2O$.

These observations led to the surmise that the barium salt as precipitated by alcohol was a true alcoholate. The truth of this hypothesis was proved by the experiments now to be described.

The Alcoholo-hydrated Barium Glycerophosphate.—A quantity of barium glycerophosphate, as prepared by the chemolysis of kephalin with barita, was precipitated by alcohol, and the precipitate washed with alcohol, after which it was exposed to the air, when it lost alcohol, became brown and somewhat brittle round the edges, and began to fuse. At this stage it was again dissolved in the minimum amount of cold water, and the solution reprecipitated by absolute alcohol. The precipitate so prepared was isolated and allowed to drain. When thoroughly drained, a small quantity was pressed in a vice between folds of blotting-paper, until it became pulverulent. It was now heated in an air-bath at 100° C., until it was approximately dry. In this way 0·7354 g. lost 0·237 g. and became 0·4978 g., corresponding to a loss of 32·30 per cent. The residue of this operation, when burnt, left a residue weighing 0·3064 g., equal to 61·5 per cent.

Pure normal barium glycerophosphate leaves on ignition 72·96 per cent. residue, while the hypothetical acid salt of formula $C_3H_7BaPO_6,C_3H_9PO_6$, might presumably leave under such conditions 65·3 per cent. of $H_2BaP_2O_7$.

A salt of the formula $\left.\begin{matrix} C_3H_7BaPO_6 \\ C_3H_9\quad PO_2 \end{matrix}\right\} H_2O$ would leave on ignition 62·9 per cent. $H_2Ba_2PO_7$.

After this preliminary experiment, the whole precipitate obtained by alcohol, amounting to 71 g., was dried by pressure between folds of paper, until it was quite pulverulent. It was then placed in 200 cc. water, in which it set like glue, but after forty-eight hours had not entirely dissolved. An addition, however, of 100 cc. more water produced a perfect solution. This was now subjected to distillation, and the alcohol contained in the first 150 cc. distillate determined. It was thus shown that in the 71 g. of glycerophosphate, which had been so dry that it could be

powdered, there were 14·84 g. of absolute alcohol, or 20·9 per cent.

The analytical results show :

Absolute ethylic alcohol - - -	20·9	
Water - - - - - - -	11·4	
Total volatile at 100° C. -	32·3	
		32·3
Residue of barium phosphate - -	41·6	(form undetermined.)
Volatile at red heat - - -	26·1	
Total glycerophosphate of Ba - -	67·7	
		67·7
		100·0

There are probably at least three molecles of alcohol and six of water combined with one molecle of acid glycerophosphate in this compound.*

The residue, from which the alcohol had been distilled, was concentrated by evaporation on a water-bath, when it deposited granular matter, which on analysis was found to be normal glycerophosphate of barium, $C_3H_7BaPO_6$.

The mother-liquor obtained after the separation of the normal barium salt just alluded to, was again precipitated by absolute alcohol. The precipitate was dried by pressure between folds of paper until it was pulverulent. It now weighed 63 g., and was dissolved in 300 cc. water, and the solution distilled to one-half. On estimation of the alcohol, it was found that the salt had contained 15·5 per cent. absolute alcohol.

Finally the residual solution of barium glycerophosphate was transformed into lead salt, and the lead salt into calcium salt. That portion of the calcium salt which was deposited from a boiling solution was found to be normal. From the mother-liquor, which grew acid, a salt was precipitated by alcohol, which, from a determination of the residue left on combustion of a portion of it, seemed to be the acid salt of calcium.

* The salts of kryptophanic acid (from urine) and kreatylic acid (from flesh) present characters which recall those of glycerophosphate of barium. Thus the copper salt of kryptophanic acid, when precipitated by alcohol, behaves like the glycerophosphate of barium already described, yielding, on distillation with water, alcohol.

The following alcoholates of inorganic salts are known : $ZnCl_2,2C_2H_6O$; $CaCl_2,4C_2H_6O$; $Mg(NO_3)_2,6C_2H_6O$; etc.

Barium glycerophosphate in a concentrated syrupy solution in water, when allowed to stand in a covered deep vessel, crystallizes, after the lapse of a very long time, in radiary masses of needles.

Glycerophosphate of barium not only seems to be the only organic compound which is known to form alcoholates, but it is also in so far unique, as it forms an *alcoholate* and a *hydrate* at the same time.

The differences observed in the relative amounts of alcohol and water may be caused by the different proportions of these bodies which are present at the moment of precipitation. If several alcoholo-hydrates are producible, the method of preparation makes it unavoidable that a mixture of these should be produced. But even if this were not the case, and if there were only one type of alcoholo-hydrate, the varying amounts of alcohol in different precipitations would compel us to assume that alcohol and water may substitute each other in indefinite proportions, as isomorphous compounds do in mixed crystals.

The insoluble lead salt is very stable; next comes the calcium salt, then the barium salt.

Other salts, such as those of silver and copper, seem to decompose at every stage of their production, so that although voluminous at first, they fall away to almost nothing during attempts at their purification.

During all transformations or concentrations of solutions, considerable quantities of the acid are decomposed, and the relative phosphates and glycerol are formed.

IV.

GROUP OF NITROGENISED NONPHOSPHORISED PRINCIPLES.

A. SUBGROUP OF THE CEREBROSIDES.

General Properties of the Subgroup.

THE cerebrosides are all white, and more or less opaque, but are capable of becoming in part transparent like wax. They are deposited from hot alcoholic solutions in minute microscopic particles, which may be termed crystalline, but have no claim to be termed crystallised. These particles are arranged in various composite forms—balls, or branched masses, or rosettes; the latter will be more fully described under the headings referring to the several varieties.

The cerebrosides are all soluble in hot alcohol, particularly absolute alcohol, and deposited on cooling; they are very little soluble in cold absolute alcohol, much less soluble, indeed, than sphingomyelin, which can thus be separated from the bulk of the cerebrins. The mixture is dissolved in hot alcohol and allowed to cool; nearly all the cerebrin bodies fall down; much sphingomyelin remains in solution. The deposit is separated from the liquid and subjected to this treatment until the mother-liquor is free from phosphorus. It is further purified, as will be shown lower down.

The cerebrosides are almost insoluble in water. One g. of purified phrenosin from ox was powdered and boiled in 100 cc. of water; the solution was filtered through force-filters, and of the filtrate 50 cc. were evaporated on the water-bath to dryness in a platinum dish. As this increased in weight by only 0·025 g., one part of this phrenosin was soluble in 2,000 parts of water. Pure phre-

nosin does not swell on being boiled with water, but remains pulverulent and unaffected. When the cerebrosides swell and become starchy in hot water, they contain phosphorised matter as an admixture.

The cerebrosides are quite insoluble in cold benzol. They swell in it, and become quite transparent, so as almost to disappear from sight. But when the benzol is filtered off and evaporated, it does not leave a vestige of matter behind. But in hot benzol the cerebrosides are extremely soluble, and on cooling are deposited as a gelatinous mass, which requires agitation before it can be filtered. From the hot solution cold alcohol precipitates white flakes. This treatment facilitates the separation of the cerebrosides from the benzol.

The cerebrosides are almost insoluble in either cold or hot ether, and are by this solvent purified from kephalin and its relations, from lecithin, cholesterin, from krinosin and istarin. But the separation from myelin and sphingomyelin cannot be effected so easily by ether as by the absolute alcohol treatment above described, the lead-process to be described, and the cadmic chloride and hydrothion in ethereal solution treatment to be described lower down.

The cerebrosides behave neutrally towards hydrothion, whether they are suspended in alcohol or ether. Any metals which may be combined or mixed with them may then be removed as sulphides without injury to the cerebrosides. They are, therefore, in this respect unlike some of the phosphorised principles, which seem to combine with hydrothion, and are with its aid able to retain metallic sulphides in solution in ether.

The mixture of cerebrosides has been repeatedly examined as to the relative amount of the elements of which it is constituted, and there have been found in percents. :

C	65·7	66·1	67·01	65·41	66·35
H	10·8	11·0	11·06	10·96	11·01
N	4·4	2·2	3·19	3·00	2·43
OP	19·1	20·7	18·74	20·63	20·21

A certain amount of sulphur is also present as a constituent of a subgroup, which will be described lower down.

The nitrogen is rarely found exceeding 3 per cent. ; so much as 4·4 per cent. has been found only once in my researches, and that in a case where the barita process had been used. In pro-

cesses in which barita was not used, the cerebroside mixture contained from 2·4 to 3·2 per cent. of nitrogen. And in a process where barita had been used, but probably in a lesser quantity, or with less effect, only 2·2 per cent. of nitrogen was retained in the cerebroside mixture.

1. Separation of the Cerebroside Principles of the Brain.

Spirit Treatment.—The white matter obtained by the process described above, from which lecithin and kephalin have been removed by treatment with ether, and which has been recrystallised from spirit eight several times, is further purified as follows :

The mixture, containing phrenosin, kerasin, cerebrinic acid, and sphingomyelin, is brought to the consistence of cream by rubbing in a mortar with spirit of 85 per cent., and is added in small quantities at a time to hot spirit in a platinum vessel. This is done in order to effect as perfect a solution of the soluble part, and to prevent as much as possible the formation of insoluble matter. The solution is filtered hot and set aside to deposit. On cooling a white body comes down and is collected. On analysis this is found to contain about 0·856 per cent. of phosphorus. This process is repeated a tenth time, and the product having lost only little of the phosphorised ingredient (which in spirit of 85 per cent. strength has the same solubility as the cerebrin bodies) is subjected to treatment with lead acetate.

Lead Acetate Treatment.—The body is triturated with *alcoholic* solution of lead acetate, and the mixture poured as before into hot spirit; the solution is filtered hot, and allowed to cool. The deposit is collected, and treated with more alcoholic solution of lead acetate, and filtered from the excess of liquid. After this it is twice dissolved in hot spirit to remove excess of lead acetate. The deposit, on analysis, is found to contain about 0·73 per cent. of phosphorus.

The body is now triturated with *watery* solution of lead acetate, and pressed to remove the excess of liquid. The mass is made into a cream with cold spirit, and added in small quantities to hot spirit. The solution is filtered and set aside to cool, and the stearoconotised portion is put aside for separate treatment. The alcoholic and watery filtrates, from which all matters deposited on cooling have been removed, are concentrated, and treated as shall be described elsewhere. The white body which comes down

from the spirit solution on cooling is collected and treated again with spirit, and this process is repeated until no more so-called stearoconote (or lead precipitate) is produced. The ultimate deposit obtained, after all these recrystallisations have been carried out, is collected and dried at 100° C. At this temperature it does not alter in appearance. It gives the purple reaction with oil of vitriol alone, and on analysis is found to contain 0·73 per cent. of phosphorus.

Lead acetate, without ammonia, as here applied, precipitates almost all true myelin, besides some cerebrinic acid and other matters. To purify the cerebrosides completely from matters of this class, it is necessary to add ammonia to the lead acetate. By that means a condition of the alcoholic solution of the cerebrosides is attained, in which neither lead acetate, nor ammonia, nor a mixture of both produces any further precipitates. Then the solution contains mainly phrenosin, kerasin, krinosin, and sphingomyelin, which are deposited on cooling, excepting only some kerasin and sphingomyelin, which remain in solution.

Absolute Alcohol Treatment without Fractionation of Precipitate.—Absolute alcohol is now used in place of spirit of 85 per cent., and the solution and recrystallisation are repeated a great number of times until the mother-liquors gives no more precipitate with cadmic chloride. As long as the alcoholic filtrate gives a precipitate with this reagent it is manifest that it removes the phosphorised sphingomyelin, and, as will be seen below, the separation of the phosphorised part succeeds, gradually but effectually, to the extent of concentrating the phosphorus in the part soluble in the cold, so that it contains nearly 2 per cent. (1·951 per cent.), while the part insoluble in the cold retains only one-tenth of that amount. But when the solubility of the phosphorised ingredient in absolute alcohol has become again equal to that of the nitrogenised substance, it is found requisite to resort to fractional precipitation for the purpose of isolating pure educts.

Separation of Phrenosin and Kerasin by Fractional Precipitation on Cooling.—When the absolute alcohol solution begins to deposit matter, which occurs between 50° and 40°, rosettes of phrenosin appear first. When the temperature reaches 28° this ceases; and the supernatant liquor is clear for a while until the temperature falls to 26°. Below this temperature a gelatinous cloudy mass, mainly of kerasin, gradually forms and floats on the phrenosin.

The phrenosin is therefore isolated as follows. When the temperature of the liquid in which the phrenosin crystallises has fallen to 28°, the mother-liquor which contains most of the kerasin in solution is swiftly decanted, either with or without the employment of a filter kept at 28° by a water-bath; and the phrenosin is thus recrystallised seven times until no further separation seems to be effected.

An analysis of a large specimen of phrenosin at this stage gave phosphorus equal to 0·182 per cent., and after two further treatments in the same way it yielded phosphorus equal to 0·113 per cent. It also gave in two estimations the following quantities of inorganic matter: (I.) =0·19 per cent.; (II.)=0·23 per cent., containing ·07 of potash (K_2O). The dried kerasin yielded phosphorus =0·198 per cent. and potash =0·07, from which it will be seen that the many resolutions and the several lead-treatments had not yet removed all inorganic ingredients from these matters.

2. Phrenosin and its Derivates.

a. Further Purification of Phrenosin by Cadmic Chloride, Ether, and Hydrothion.

The phrenosin isolated by fractional precipitation as described above is mixed with solution of cadmic chloride, and the mixture suspended in ether. Hydrothion is passed into this, when a yellow solution and a yellow precipitate form, which remain mixed with the bulk of the undissolved phrenosin. The solid matter is filtered from the liquid; it consists of phrenosin and cadmic sulphide, while a peculiar compound of a phosphorised body with cadmic sulphide remains in solution. The residue is removed from the filter and dissolved in hot 85 per cent. spirit. The insoluble cadmic sulphide is filtered off, and the solution is set aside to deposit. On cooling phrenosin comes down in large rosettes, and is collected and recrystallised, first twice from 85 per cent. spirit, and finally from absolute alcohol. It is dried, and then exhibits the following properties. When boiled with water it does not swell to a starchy paste, but merely becomes flocculent and floats about in the fluid. Treated by itself in the cold with oil of vitriol it very slowly develops a purple reaction, but more quickly when warmed. It passes through an intermediate yellow stage, particularly well marked in the reaction, which is obtained without the employment of heat, and during which

the matter is wholly in solution. Then flocks separate and slowly become purple.

Two quantations of phosphorus were made : No. 1 gave 0·045 per cent., and No. 2 gave 0·051 per cent. Taking the mean of these two results, we have 0·048 per cent. of phosphorus. Calculated as myelin, this would give 1 per cent. as the amount of phosphorised substance left in the phrenosin.

At this stage of its purification the phrenosin was subjected to elementary analysis.

Elementary Analysis of Phrenosin, $C_{41}H_{79}NO_8$.—Carbon, hydrogen and nitrogen were determined simultaneously by the vacuum method. The substance was burnt with oxide of copper and copper in vacuo, the resulting water was weighed, and the gaseous mixture of carbonic acid and nitrogen was analysed and estimated volumetrically.

The nitrogen was further determined by the methods of Liebig and of Dumas as modified by Thudichum and Wanklyn, and by the proceeding of Will and Varrentrapp. The percentages obtained in these analyses are compared in the following table :

	By combustion in vacuo ; C and N volumetrically ; H_2O weighed.			CO_2 wgd.	By combustion in CO_2 atmosphere; gas volumetrically estimated.			By combustion with soda-lime ; Pt salt weighed.		
	a.	*b.*	*c.*	*d.*	*e.*	*f.*	*g.*	*h.*	*i.*	*k.*
C	67·71	67·89	67·37	68·56						
H	11·62	11·42	11·23							
N	2·15	2·13	2·07		2·29	2·18	2·34	1·768	1·690	1·715
O	18·51	18·56	19·03							
	100·00	100·00	100·00							

Consideration of the Methods of Analysis.—The quantation of the nitrogen as gas, whether it has been obtained by combustion in a vacuum or by combustion under atmospheric pressure in an atmosphere of carbonic anhydride, always gives the nitrogen a little too high as compared to theory, whereas the estimation of nitrogen by transformation into ammonia always gives the amount of this element a little lower than is required by theory. These discrepancies are well known to be inherent in the methods. They are less significant as regards the analysis of bodies which are rich in nitrogen, than in the analysis of bodies containing only a small percentage of this element. In the particular case of

phrenosin they are so great as to make it at first sight impossible to derive a correct empirical formula from the data given by either mode of analysis. But *the mean* of the data for nitrogen obtained by the three different modes of analysis is very nearly coincident with the sum of the data obtained by the chemolytic method. This coincidence is probably the result of accident only, at least there is at present no explanation derivable from the most searching scrutiny of either of the methods employed, or of the particular manner in which they have been executed.

In the vacuum analysis the carbon is regularly found a little too low; yet not a trace of carbon is left unburned in the tubes, as was specially proved; moreover, the combustion tubes with their contents were weighed, before and after combustion, and the weights of the sums of the products of combustion, as calculated from their volumes in the case of carbon and nitrogen, showed an almost mathematical coincidence with the respective losses which the tubes had undergone.

In this case of phrenosin the known methods of elementary analysis are therefore unavailing to lead to final results; on the contrary, phrenosin is a good test object by the use of which the particular failings as well as strong features of these methods can be made apparent. Thus, combustion, according to Liebig, of not too small a quantity of phrenosin yields the best carbon estimate; combustion in vacuo of such a small quantity as that to which the method is necessarily limited, the worst.

The very same methods which in the case of phrenosin yield the results discussed in the foregoing, give, when applied to its decomposition-products, results which are in much greater concordance with the requirements of theory. This unquestionable fact shows that the size of the molecle, and the proportions in which the elements contained stand to each other, have an influence on the result of the process of elementary analysis; a simple compound of fewer atoms gives more accurate results when tested by different methods, while a more complicated compound with many atoms, when tested in the same manner by different methods, gives much less accurate results. It is in accordance with this that the elementary analyses of the more complicated nitrogenised bodies, such as kerasin and cerebrinic acid, present even greater difficulties than those which have been experienced in the analysis of phrenosin.

b. Chemolysis of Phrenosin by Sulphuric Acid in Watery Solution.

Introduction.—In my earliest chemolyses with barita, hermetically sealed glass tubes, enclosed in iron tubes, were employed. But of these at least half succumbed to the internal pressure, and their contents were lost. I therefore procured a special tube of brass lined with platinum. This worked satisfactorily for the barita chemolyses, which were effected in a short time; but the sulphuric acid chemolyses were found for their completion to require the influence of a temperature of at least 130° for a period varying from 310 to 370 hours. It was therefore necessary to multiply the tubes, and in order to do this consistently with practical considerations leaden tubes were employed. Such tubes could not have been employed in the barita chemolysis, as barita rapidly and energetically attacks lead. But dilute sulphuric acid has but a slight influence on metallic lead, and the small quantity of metal which is dissolved is easily removed from the organic products by appropriate means.

The Apparatus.—The Leaden Tubes.—The Hot-Air Stove.—The leaden tubes are an inch in calibre; the metal is an eighth of an inch thick; each tube is eighteen inches long. One end of the tube is closed by hammering only so as to form a semi-globular end, not larger in diameter than the tube itself; it is tested by water, and when none passes it is soldered over on the outside. The mixture to be chemolysed is now put into the tube by means of a wide-tubed funnel; the upper part of the tube is heated to dry it completely and rarefy the air in the air-space, which amounts to about one-sixth of the length of the tube; the mouth of the tube is now suddenly compressed in a vice and closed; the edges of the lead are filed smooth, moistened with zinc chloride, and immediately soldered, or, as the operation is technically termed, 'burnt,' with the oxyhydrogen flame.

The hot-air stove is made of copper, and consists of two horizontal air cushions, between which special room for six of the leaden tubes just described is arranged so that their longest axes are lying in a horizontal position. The tubes can thus be kept at an equable temperature, which can be read by the thermometer, the ball of which is in the central air-space, while the stem projects over the top of the stove. The stove is heated by aërated gas flames.

Preliminary Purification from Inorganic Salts of the Phrenosin to be Chemolysed.—As phrenosin retains inorganic salts with great pertinacity, it must be subjected to a process for the removal of these. It is boiled in water until completely disintegrated, and the boiled mixture is pressed through a cloth. The filtrate is then mixed with sulphuric acid sufficient for it to contain 1 per cent., and boiled for one hour. The phrenosin is soon curdled out of the solution, free from salt but partially altered. For the acid solution after treatment with barita carbonate is found to contain some chemolytic sugar (cerebrose) besides some alkaloidal matters, and the potassium, sodium, and earthy salts which it is the object of the process to remove.

Chemolysis of the Purified Substance.—Six leaden tubes, prepared as above, receive each about 30 g. of nitrogenised substance purified as described, and 353 cc. of dilute sulphuric acid containing 2 per cent. H_2SO_4. The tubes are then closed as described, placed in the hot-air stove, and heated to 130° during twenty-four hours. After the lapse of this period the tubes are opened at the compressed end with a chisel, the dilute acid is removed and filtered, all solid matter is kept in the tubes or returned to them together with a fresh charge of acid; the tubes are again closed as described, and heated for a second twenty-four hours. This treatment is repeated as long as the acid liquid contains any cerebrose, and the chemolyses are deemed complete only when the last charge of dilute acid is found on proper treatment and concentration to be free from cerebrose. This result is in most cases not attained in less than fourteen days, and in some requires from sixteen to seventeen days, in a few even twenty-four, during which the chemolysis is continued day and night.

The Acid Filtrates.—These are boiled with barium carbonate, prepared pure by precipitation for the purpose. The neutral filtrates are evaporated to about one-fifth of their bulk in a water-bath, then removed into a distilling apparatus connected with an air-pump, and distilled at a temperature of from 30° to 40° to the consistency of a syrup. The latter is put aside to crystallise. Stellate groups of crystals soon form, and the entire syrup gradually solidifies to a granular mass of crystals. These are separated partly by drawing off the small quantity of mother-liquor, partly by agitating them with water, and getting rid of

the mother-liquor by dilution; for the crystals are but slowly soluble in cold water.

From the mother-liquor a further quantity of crystals is obtained by addition of boiling alcohol to the hot concentrated aqueous solution until a considerable permanent turbidity is produced. When the mixture is allowed to stand in the cold for one day, it forms a considerable amount of a coloured deposit, from which the clear supernatant alcoholic liquid is decanted. This latter solution on standing for some weeks, and after repeated additions of small quantities of alcohol, and lastly of ether, deposits a crop of white crystals which are added to those obtained in the first operation.

The mother-liquors are, however, always considerable in amount, and after concentration yield an uncrystallisable syrup, which amounts to about the same weight as the crystals obtained from it. The syrup can be made to crystallise a third time after the removal of some traces of potassium and of a compound ammonium base by platinic chloride, and the removal of all traces of the reagent by hydrothion and silver carbonate.

c. *Cerebrose, a New Crystallised Sugar.*

The Crystals.—Cerebrose $C_6H_{12}O_6$.—The crystals obtained as described in the foregoing are dissolved in water, and the solution is evaporated in vacuo, after treatment with animal charcoal. A mass of crystals is again obtained, which are smaller than the first ones, but perfectly white and very hard. The colourless mother-liquor of these on standing over oil of vitriol solidifies to a hard mass of crystals, which rise much over the level of the liquid in which they form. The crystals are not large enough for crystallometric treatment; seen under the microscope they seem to consist of rhombic octahedra, of which some are elongated to prisms. It is a kind of sugar, to which I have given the name of *Cerebrose.* On elementary analysis it gives analytical data, leading to formula $C_6H_{12}O_6$.

Reducing Power of Cerebrose over Cupro-Potassic Tartrate.—Cerebrose reduces Fehling's solution readily on heating, and the precipitated suboxide of copper is mostly of a dark-red colour. 0·2622 g. cerebrose dried in the water-bath were dissolved in water, and the solution made to fill the space of 50 cc. This solution therefore contained 0·5245 per cent. of cerebrose. It

was employed to reduce a Fehling's solution of which 5 cc. required 0·025 g. of glucose for decoloration. Five cc. of the Fehling's solution required 5·6 cc. of the cerebrose solution for decoloration, equal to 0·0294 g. cerebrose. A certain quantity of cupro-potassic tartrate, therefore, which requires five parts of glucose for complete reduction, requires about six parts of cerebrose for reduction.

Polarising Power of Solution of Cerebrose.—Some cerebrose was dried in the air-bath at 90°, and in vacuo till it remained constant in weight. Of this 2·6315 g. were dissolved in water with the aid of a gentle heat, and made to fill a space of 20 cc. The solution was treated with animal charcoal to remove a slight turbidity. Its strength remained at 13·16 per cent. This in a tube of 100 mm. length rotated the ray of polarised light at 25° T = +10° 40′; but after twenty-four hours' standing the same tube at T 11° rotated only + 9° 24′; after a second twenty-four hours, at T 12° = + 9° 18′. The solution was then diluted with an equal bulk of water, and its rotation, measured in a tube twice the length of the former, was found to be + 9° 24′ at 9° T to + 9° 32′ at 8° T. These data, by means of the usual calculation, lead to the specific or limited rotation for cerebrose of +70° 40′. It will be observed that immediately after solution the rotation is a little higher than twenty-four hours after solution, when it becomes constant. But this increase of the rotation is very slight compared with the increase which dextroglucose exhibits immediately after solution, and which, from the fact of its being about as much again as the constant rotation, is termed the birotation of glucose.

The molecular formula of cerebrose is assumed in the foregoing to be $C_6H_{12}O_6$. Cerebrose resembles sugar of milk by its feebly sweet taste and the great hardness of its crystals.

Other Properties of Cerebrose.—The crystallised cerebrose is never obtained without the amorphous modification being formed at the same time. To judge from comparison of bulks, at least half the cerebrose obtained during the chemolytic operations on the nitrogenised principles passes into the amorphous state, and cannot be made to crystallise entirely even within the period of a year. Crystalline particles of pure cerebrose immersed in the syrup increase in size, and form ramifications; but their growth ceases after a time, and the syrup thereafter remains unchanged. The difficulty of separating the two modifications is increased by the

occasional appearance of a third form of product of the chemolytic metamorphoses of the amylonide radicle of the nitrogenised bodies which I shall presently describe.

Cerebrose is precipitated from its watery solution by basic lead acetate or by a mixture of neutral lead acetate and ammonia; the mother-liquor of this precipitate no longer reduces potassio-cupric tartrate, from which it may be inferred that the precipitation of the cerebrose is complete. The lead compound of cerebrose after decomposition by hydrothion yields the cerebrose in the free state. In its affinity for lead oxide cerebrose resembles inosite, the sugar naturally contained in the brain, and obtained as an educt from the water extracts; but it is easily distinguished from inosite by its power over polarised light and potassio-cupric tartrate, reactions which inosite does not possess.

The Uncrystallised Cerebrose.—The uncrystallised cerebrose obtained from the nitrogenised substances by the process of chemolysis above described may be a product from the crystallised; at least, when the watery solution of the sugar is evaporated in the open air on the water-bath no crystallised sugar is ever obtained, but, as previous experience and renewed experiment have shown, only uncrystallisable cerebrose. Only when the solution is evaporated in a vacuum ensuring the absence of air, and at a temperature never rising above that of the animal body, 37°, is crystallisable cerebrose obtained—accompanied, however, always by a considerable proportion of uncrystallisable cerebrose, amounting in weight to about that of the crystallisable cerebrose. There is, therefore, room for an inquiry into the causes of these phenomena.

The chemical constitution of cerebrose now arises as a subject of inquiry of interest and importance. Not only is there a new isomer added to the long list of saccharoid substances already known, but, what is of much greater value, a new key is found to the knowledge of the constitution of some of the organoplastic substances. This will enable us to obtain a full knowledge of the constitution of the nitrogenised substances of the brain much quicker than would be the case without such theoretical aid; for the number and nature of the problems are now at once limited and defined, as we shall see presently by the aid of further new data.

Cerebrosic Acid, $C_6H_{10}(H_2)O_6$.—This new acid, obtained by

means of the chemolytic process from phrenosin, has the composition of a carbohydrate, and is probably isomeric with cerebrose. It has not yet been examined any closer in the free state, but the examination of its barium salt leads to the inference that it is a dibasic acid of the formula $C_6H_{12}O_6$. It is obtained as follows: 25 g. of pure phrenosin are suspended in 300 cc. of water, and to the mixture 2 cc. of oil of vitriol are added. The whole is enclosed in the platinum chemolyser, and heated to 120° during seven days without interruption. The acid liquid from the chemolyser is now filtered and treated with barium carbonate at the boiling heat. The filtrate (which in the chemolyses of phrenosin, where the dilute sulphuric acid is renewed every twenty-four hours, mainly contains cerebrose) has no reducing effect at all upon potassio-cupric tartrate, but contains a considerable amount of barium in solution. When evaporated quickly to dryness, it leaves as residue a hard amorphous barium salt; but when dissolved in a little spirit and allowed to stand, it slowly sets into a mass of indistinct crystals. These are freed from mother-liquor by pressure between folds of bibulous paper dried at 100° C., and analysed with the following result:

Synopsis of Analyses of Barium Cerebrosate.

Elements.	Percentages.	÷ At Wts.	÷ Ba. = 1.
C	24·53	2·0442	6·40
H	3·20	3·20	10·09
Ba	43·50	0·3175	1·00
O	28·77	1·798	5·66

These data lead to a formula $BaC_6H_{10}O_6$, corresponding to an acid of the composition of cerebrose in which two atoms of hydrogen are replaced by an atom of barium. It reminds of glucic acid, which is also dibasic. Glucic acid is prone to form bodies like the caramels, and the presence of a small proportion of one or other of these bodies seems to be the cause of an excess of carbon over the theoretical amount which is met with in the analysis of its salts. A similar feature is observed upon the cerebrosate of barium, which also exhibits a slight excess of carbon and a deficiency of hydrogen and oxygen. The salt is free from nitrogen, and does not contain sulphuric acid in organic combination. When distilled with phosphoric anhydride it does not give out the odour of acrolein, but a smell resembling

burnt sugar. With oil of vitriol alone it gives no purple reaction. Mixed with sphingosin and oil of vitriol it gives a brilliant purple reaction, equal in tint to that produced by the aid of cerebrose. But even its concentrated solution has no influence on the alkaline copper solution.

Transformation of Barium Salt of Cerebrosic Acid into Zinc Salt.—The barium salt is decomposed with dilute sulphuric acid, and the solution extracted with ether. The ether, on distillation, leaves an acid which is converted into zinc salt: the latter is crystallised and analysed. The air-dried salt loses 9·8 per cent. of water at 100° C. The remaining dry salt is burnt, and the zinc estimated, from the remaining oxide, to amount to 28 per cent. of air-dried salt. From these data it seems that the zinc salt is entirely different from any of the known lactates. The barium salt, though different in appearance from lactate, is isomeric with it.

Attempt to produce Cerebrosic Acid from Free Cerebrose.—A consideration of the conditions under which this acid has been produced from phrenosin suggests that it might have been formed by the prolonged action of heat and acid upon cerebrose formed during the earlier stages of the reaction. In the experiments in which cerebrose was obtained, the influence of acid and heat upon the phrenosin was interrupted every twenty-four hours, and the acid was renewed. In the experiment, however, which yielded cerebrosic acid, the action of acid and heat had been continuous during seven days and nights. It was therefore necessary to investigate whether cerebrose already formed could be transformed into cerebrosic acid under the circumstances related, or whether it could be so transformed only in the nascent state, and what were the other conditions of this transformation.

About 2 g. of amorphous cerebrose were heated with water containing 1 per cent. of oil of vitriol, to 120° during nine days. There were formed, firstly, a considerable amount of caramel; and secondly, a quantity of an acid corresponding, as regards its properties and those of its salts, to cerebrosic acid. But a large proportion of the cerebrose remained unchanged. The caramel was, like the acid, soluble in ether.

Caramel obtained in the Chemolysis of Phrenosin in which Cerebrosic Acid was formed.—Of this caramel 2·1 g. were obtained. It was very soluble in ether, and somewhat soluble in alkaline water, but insoluble in alcohol and in acidulated water. It was of a deep

brown colour, like all the bodies of this class. From a consideration of the several forms of caramel which are obtained from the several principles of the nitrogenised group, as will be shown in another chapter, it becomes probable that the caramel here formed was, in part at least, the caramel of psychosin. This supposition is strengthened by the consideration of the relative quantities of the several products of the chemolysis of phrenosin. The 25 g. of this body employed in the experiment related above gave 4·6 g. of cerebrosic acid. The theoretical amount which could have been obtained in the best case would have been 6·2 g. Consequently 1·6 g. of the amylonide radicle must have been transformed into bodies other than cerebrose and cerebrosic acid. The 2·1 g. of caramel found in the present experiment could not have been caramel of cerebrose simply, as the atomic weight of that body is much smaller than that of cerebrose. But it could have been, or contained, some caramel of psychosin, the atomic weight of which is more than twice that of cerebrose. The other products insoluble in water were, sphingosin, which as sulphate weighed 2·5 g., corresponding to about 2·13 g. free sphingosin, and an unascertained quantity of another alkaloid, probably psychosin, which remained in the alcohol from which the sphingosin was precipitated by sulphuric acid. The amount of free fatty acid obtained was 9·4 g. This was almost entirely neurostearic acid, and its quantity came very near to that required by theory, which is 9·9 g. The total of weighed products of chemolysis amounted to 18·2 g.; the psychosin or second base was not weighed, and allowing for this, certainly a few grammes, and much for loss in the many difficult manipulations, the fate of the phrenosin originally employed is pretty well accounted for.

In almost all chemolyses of nitrogenised principles by acids or alkalies in watery or spirituous solution there has been formed cerebrose, cerebrosic acid, psychosin, caramel of psychosin, sphingosin, and neurostearic acid or its ether. When the cerebrose solution was freed from sulphuric acid, after chemolysis with an acid, it always retained some baryta, which had to be removed by precipitation with sulphuric acid. It is therefore possible that the cerebrose is always accompanied by some cerebrosic acid; attempts should be made to extract this by ether before evaporating the cerebrose solution to a small bulk, as otherwise

the acid may contribute to transform the cerebrose into the uncrystallisable modification.

d. Sphingosin, a new Alkaloid, as Sulphate, and Fatty Acids.

The solid products from the chemolytic tubes are united, edulcorated with water, dissolved in hot spirit, decolorised with animal charcoal, crystallised and dried. In a state of fine powder they are extracted with pure ether in the cold. The fatty acids dissolve, while a body remains insoluble, which is of an alkaloidal nature, and to which, in commemoration of the many enigmas which it presented to the inquirer, I have given the name of *Sphingosin.*

The part insoluble in ether is again treated with alcohol, but the substance, previously freely soluble in spirit, now becomes more and more insoluble, and at last fuses and becomes quite insoluble even in absolute alcohol. It is now easily soluble in benzol in the cold. Addition of any acid to the hot alcohol restores the solubility of the body, and on cooling the body crystallises again. This bearing leaves little doubt that the body is a salt-like combination of an organic base with the acid employed, soluble in alcohol in the presence of an excess of acid, insoluble in the absence of such excess.

Removal of the Sulphuric Acid by Caustic Alkali.—The salt is freed from spirit by water, and then while diffused in water is treated with caustic soda ley and heated. The flaky body at once transforms into oily drops, which rise to the surface of the liquid. On cooling this oily liquid does not set like a fat, but becomes again opaque, and distributed in flakes through the fluid. The oil is, however, easily soluble in pure ether (in which the salt had been previously insoluble), and is extracted by this solvent. The solution is filtered, the ether distilled off, the residue dissolved in absolute alcohol and decolorised by animal charcoal. Minute quantities of impurities, probably of soda-soap, which deposit from the ether solution and from this last alcoholic solution on standing, are removed by filtration. This alcoholic solution of the free base, which is alkaline to test paper, gives the following reactions. Oil of vitriol in absolute alcohol gives an immediate white precipitate of a sulphate. Hydrochloric acid gives a precipitate of a hydrochlorate; both precipitates are soluble in cold alcohol with the aid of excess of acid. An alcoholic solution of cadmic chloride gives a precipitate soluble in excess of absolute

alcohol. Mercuric chloride gives a flaky precipitate which settles easily. Water causes a gelatinous precipitate in the alcoholic solution. Ether or alcohol solution leave the base in a crystalline state on evaporation. It is very slightly if at all soluble in water, even on boiling. When dry it gives no purple colour with oil of vitriol alone on gentle warming, but on addition of sugar gives immediately a deep purple colour. The full significance of this reaction will be discussed lower down.

Sphingosin Sulphate.—A solution of the free base in cold absolute alcohol is precipitated with a freshly made solution of oil of vitriol in absolute alcohol with the precaution of keeping the alkaloid in excess. The white crystalline precipitate is washed with absolute alcohol, pressed, and dried in vacuo over sulphuric acid.

Elementary analysis leads to the formula for sphingosin sulphate of $2(C_{17}H_{35}NO_2)+H_2SO_4$, of which the theory is compared with the experimental data in the following table :

Theory of: Atoms.		Theory of: Percents.	Found. Percents.
34 C	408	60·11	60·85
72 H	72	10·78	10·70
2 N	28	4·19	4·14
4 O	64	—	—
S	32	14·37	14·32
4 O	64		
	668		

Sphingosin Hydrochlorate.—On adding to a concentrated solution of sphingosin in absolute alcohol or in spirit some hydrochloric acid, a turbidity or precipitate is at once produced. If the mixture is warmed and allowed to cool gradually under a dryer over oil of vitriol, masses of spear-shaped crystals form in the fluid which can be removed as a felted mass. The crystals under the microscope appear as uniform needles with pointed pyramidal ends. The analytical data concerning this salt show that its formula is $C_{17}H_{35}NO_2+HCl$. It does not easily form double salts with metallic chlorides.

The purification of sphingosin is based upon its precipitation from absolute alcohol by sulphuric acid and the decomposition of the sulphate in water by caustic alkali ; the oily mass when freed

from all alkaline liquid can be boiled with water, and thus freed entirely from alkali. In this way two impurities which may accompany sphingosin in small quantities are entirely removed; one a body soluble in ether, and which will be described hereafter; another an acid of which also a small quantity escapes the first extraction by ether, in which the bulk of the acid formed in the chemolysis is separated. Both remain in the absolute alcohol from which the sphingosin is precipitated by sulphuric acid. But they are less soluble in watery alcohol than the sphingosin, and therefore are precipitated by water or watery reagents together with the sphingosin.

Consideration of the General Chemical Function of Sphingosin.—The consideration of the molecular formula of sphingosin might at first sight lead to the hypothesis that it contained a fatty acid radicle of the $C_nH_{2n}O_2$ series, and that in this an atom of hydrogen was replaced by the amide group NH_2, as expressed by the formula $C_{17}H_{33}(NH_2)O_2$. Sphingosin also behaves like an amido-acid in this, that on the one hand it combines with bases such as potash, or on the other unites with acids such as sulphuric and hydrochloric. However, its qualities as an acid are the least apparent, and so limited that they subsist in any degree only in the absence of water. In the presence of water the potash compound is not formed at all; the barium compound is formed in the presence of water and excess of barium salt, but decomposes during every treatment for its purification, even by resolution in strong spirit. The salts of sphingosin with acids, however, are very firm compounds; they crystallise, do not dissociate in solvents, and in the dry state admit of convenient manipulation. By its greater affinity for acids, sphingosin indeed differs from the amido-acids and resembles more the alkaloids. It is precipitated by most of the specific reagents which combine with alkaloids, and therefore on the whole evidence it must be admitted that sphingosin is an alkaloid.

Neutral, Acid, and Basic Salts; Bearing of the Sulphate.—When sphingosin sulphate is treated with dry neutral ether, it remains undissolved. When the mixture is acidified with sulphuric acid, the sulphate dissolves completely. When to the solution some alkali is cautiously added, the neutral sulphate is again precipitated in flocks. When neutral sphingosin sulphate which has been dried completely is digested in the cold with aqueous

ammonia, sulphuric acid is dissolved, and appears in the filtrate. But it is not practicable to remove all the sulphuric acid from the sphingosin by this treatment; even after many days' washing the alkaloid retains some sulphuric acid. This can only be removed by warming the compound with caustic ley, and extracting the free alkaloid with ether.

It follows from the foregoing that sphingosin forms neutral and acid, and perhaps basic, salts.

Bearing of the Hydrochlorate.—The hydrochlorate of sphingosin crystallises from water or alcohol in long spear-shaped crystals. It is much more soluble in hot water than in cold; it is deposited from a solution in water which also contains psychosin almost entirely if the solution is cooled to and filtered at the temperature of melting ice. A little psychosin, however, easily remains with the sphingosin, so that in analyses of the latter the carbon is sometimes found a fraction of a per cent. too high. The admixture is recognised by the purple test with sulphuric acid, and is removed by recrystallisation of the hydrochlorate; or in case of the sulphate, by transformation into the free base, and reprecipitation from absolute alcohol by sulphuric acid. The psychosin salts of both acids are the more soluble ones.

Sphingosin with Potash.—When the solution of free sphingosin, prepared from the pure sulphate in ether, is dried by being allowed to stand over solid caustic potash, a dense but translucent deposit in the shape of flakes and crusts, covering the sides of the vessel and the masses of potash, is gradually formed. This is a compound of sphingosin with potash, which is little soluble in the anhydrous ether. It has not yet been separated from the excess of potash. The ether retains a certain portion of this compound in solution, and leaves it as a hard, dense, colourless deposit on distillation. A preliminary analysis of this residue gave 61·04 per cent. carbon, 9·96 per cent. hydrogen, 4·52 per cent. nitrogen, and 6·53 per cent. potassium. From this it is evident that this residue is a mixture of a compound formed of sphingosin and potassium or potash, with free sphingosin. The potassium compound $C_{17}H_{34}KNO_2$ requires 12·1 per cent. of potassium. The mixture therefore contains a little more than half its weight of the potassium compound.

Separation of Psychosin from Sphingosin.—This may be effected in alcoholic or watery solution. The mixed alkaloids are dissolved

in absolute alcohol, and a very dilute solution of oil of vitriol in absolute alcohol is added so as to precipitate all sphingosin. From the filtered alcoholic mother-liquor all alcohol is removed, first by distillation, afterwards by evaporation with water. The residue is warmed with some caustic potash, and the ley which has taken up any sulphuric acid is decanted. The washed residue is boiled with hydrochloric acid, in which it dissolves readily. The solution is filtered hot, and concentrated to a suitable bulk. It contains all the psychosin, and if the precipitation of sphingosin by sulphuric acid had been incomplete, or an excess of the acid had been added so as to redissolve some sphingosin as acid sulphate, this base also occurs in the hydrochlorate. The solution, on being cooled to 0° C., deposits sphingosin hydrochlorate in colourless crystals, which may be filtered off in the cold, but cannot be washed from excess of psychosin by water, as they swell and make filtration impossible.

Fatty Acids and Matters soluble in Ether, being Products of the Chemolysis which yields Sphingosin.—The ether solution of the chemolytic products obtained as described above, p. 149, is concentrated, and yields several deposits of fatty matters at several stages. The first deposit after recrystallisation from spirit resembles neurostearic acid in appearance. It begins to fuse at 73°, but does not wholly fuse till the temperature reaches 81°. The body is therefore probably neurostearic acid, mixed with a small quantity of acid of lower fusing-point. It is converted into barium salt; this is extracted with hot spirit, and the fatty acid is again extracted from the barium salt by tartaric acid and ether. The free acid now fuses at 80° and sets at 79°; it has therefore lost some of the fatty acid melting at a lower temperature.

The later deposits from ether and all the acids most soluble in ether are converted into barium salts and exhausted with boiling alcohol. There remains insoluble in spirit a barium salt, which by treatment with tartaric acid and ether gives free fatty acid which fuses below 50° C., and therefore differs greatly from the acid first deposited from the ether solution.

The alcoholic extracts of the barium salts on cooling deposit a *solid compound*, and retain in solution a compound which, after evaporation of all alcohol, is *semi-solid* on being heated with water, and dissolves entirely in ether.

We therefore obtain four principal bodies by these operations on the chemolysed matters soluble in ether. Two form barium salts insoluble in boiling spirit, and are—the first, mainly neurostearic acid, $C_{18}H_{36}O_2$; the other, an acid of lower fusing-point, not yet studied any further. Of the bodies soluble in hot spirit, one is soluble in ether. The significance of all these bodies will be made clear when we come to consider the chemical constitution of the non-phosphorised group of nitrogenised substances. Each of these acids, which by their number no less than their properties are remarkable, will then find a place in a natural educt, and by another process in a systematic classification.

e. Psychosin, its Properties and Metamorphoses.

This alkaloid was first obtained from the nonphosphorised group of nitrogenised bodies by chemolysis with caustic barita. The mere analysis of this body led to an empirical formula of $C_{25}H_{49}NO_8$, which was uncontrolled by combinations. The free body had, however, crystallised from alcohol. To fill up this void I produced some combinations with hydrochloric acid and with platinic chloride, but the products had the peculiarity of some of the phosphorised bodies, namely of dissociating in the presence and under the influence of small quantities of water. The hypothetical salt would have contained 24·01 per cent. of platinum, whereas the actual salt contained only 21·05 per cent. But the carbon of the organic molecle decreased slightly in relation to the nitrogen, and this led me to again analyse a further purified specimen of psychosin, obtained by the chemolysis of phrenosin.

The psychosin was dissolved in hydrochloric acid and water, and the solution made perfectly bright and colourless. The alkaloid was then precipitated by ammonia, washed perfectly, dried in vacuo, and analysed.

Summary of Analyses of Psychosin.

	First Analyses (1876).	Later Analyses (1878).		
	Percents.	Percents.	÷by At. Wgts.	÷by N=1.
C	61·86	61·32	5·11	23·53
H	10·09	10·09	10·09	46·46
N	2·88	3·04	·21716	1·00
O	25·17	25·55	1·597	7·35

Psychosin Sulphate.—Psychosin is soluble in very dilute sulphuric acid on boiling, but is deposited on cooling. The mother-liquor

of the deposit after twenty-four hours retains only a small amount of the salt in solution, so that ammonia, which if not added in excess precipitates psychosin from its solution in water and hydrochloric acid, hardly causes any precipitate. But phosphomolybdic acid causes a more appreciable precipitate.

Psychosin Hyrochlorate.—When to a solution of neutral psychosin hydrochlorate in water an excess of strong hydrochloric acid is added, a bulky gelatinous precipitate, much resembling hydrate of alumina, is produced. The salt is so completely removed from the solution that the latter, after filtration, gives no precipitate with phosphomolybdic acid.

When a solution of psychosin hydrochlorate is kept on a dialyser of parchment-paper floating on distilled water for eighteen hours, some psychosin as well as some hydrochloric acid pass into the water. The amount which passes is very small, and no psychosin is deposited on the dialyser. Psychosin is therefore a strong base, but at the same time it exercises its functions as a colloid, such as it becomes in the presence of water and absence of acids.

Psychosin precipitated from its hydrochloric solution by ammonia and well washed, when allowed to remain in contact with pure water during several days, becomes hydrated, and swells up to a voluminous gelatinous mass. This paste retains water with great force, and is most difficult to dry in vacuo over sulphuric acid. Heat causes it to become brown at once; even in the vacuum it assumes colour.

Psychosin and Ammonia.—Psychosin dissolves readily in concentrated ammonia-water on boiling, and is deposited again on cooling, and standing. The hot ammonia solution gives a precipitate with barium chloride, which after isolation is soluble in boiling alcohol and deposited on cooling. This compound loses barium by all attempts at recrystallisation.

Chemolysis of Psychosin by Dilute Sulphuric Acid.—Psychosin is enclosed in the platinum chemolyser with a sufficiency of sulphuric acid of 2 per cent. strength, and heated to 130° during forty hours. The sulphuric acid solution, separated from the insoluble part, boiled with barium carbonate, etc., reduces Fehling's solution. The solution also contains a small amount of an acid similar to the cerebrosic acid described above. The cerebrose obtained

amounts to less than half the theoretical quantity, but is supplemented by a small portion of its derivate.

The solid products of the chemolysis are coloured brown; when warm they are in a state of semifusion. The mass is gently warmed with soda solution and shaken with ether. It dissolves in the ether without residue, imparting to it a deep brown colour. The ether is distilled off and the residue treated with hot absolute alcohol. This solvent leaves a small quantity of caramel of psychosin as a brown mass, and dissolves a quantity of slightly coloured matter. The solution is decolorised with animal charcoal, and precipitated with a little sulphuric acid dissolved in absolute alcohol. The precipitate is isolated, washed with ether, and dried. It gives no purple reaction with oil of vitriol alone, thus proving the absence of psychosin, but with a solution of cane-sugar or cerebrose the sulphuric acid solution gives a brilliant purple reaction. The precipitate is therefore sphingosin sulphate.

The alcoholic mother-liquor of the sphingosin sulphate contains some matter in solution, which gives, after removal of the alcohol, a purple reaction with oil of vitriol by itself. It is some psychosin which has resisted the chemolytic action of the dilute sulphuric acid.

The chemolysis of psychosin by sulphuric acid therefore takes place according to the equation :

$$\underbrace{C_{23}H_{45}NO_7}_{\text{Psychosin.}} + H_2O = \underbrace{C_6H_{12}O_6}_{\text{Cerebrose.}} + \underbrace{C_{17}H_{35}NO_2}_{\text{Sphingosin.}}$$

Purple Reaction with Oil of Vitriol.—I have shown that all the cerebrosides give with oil of vitriol, on gentle warming, a reaction, which consists in the formation of a deep purple colour. This reaction, which, when produced with cane-sugar and oil of vitriol, is known as Raspail's or Pettenkofer's reaction, and was for a long time believed to be specific to biliary acids, has been shown to be common to a large number of bodies which probably have a radicle in common. But most of these bodies, *e.g.* oleic acid, require the addition of sugar, and do not give the purple with oil of vitriol alone. Now, the known cerebrosides are distinguished from the biliary and fatty bodies by the faculty of giving the purple reaction under two different sets of conditions. They give it with oil of vitriol alone, on gentle warming, after a little time of standing; and they give it quicker if with the oil of

vitriol a little sugar is at once added. Now we know that all these bodies contain the radicle of the sugar cerebrose, which, as I have shown, gives with pure glykocholic acid and sulphuric acid a brilliant Pettenkofer reaction, and is therefore capable of replacing cane-sugar to its full value in this process. It is therefore clear that the ability of the cerebrin bodies to give the purple with oil of vitriol alone has for one of its causes the presence in their constitution of the radicle of cerebrose. That they react slower with sulphuric acid alone than with sulphuric acid and sugar added is perhaps explained by the facts evolved in the chemolysis above described, namely, that the splitting off of cerebrose from the other radicles requires time.

We are now able to advance the hypothesis, which has a high degree of probability, that every phrenosin-like body which gives a purple reaction with oil of vitriol alone contains the radicle of cerebrose besides that other radicle which, with any sugar, cerebrose, or cane-sugar, gives the purple, and which we will term the oleo-cholide radicle. On the other hand, any phrenosin-like body which does not give the purple with oil of vitriol alone may contain either the cerebrose or the oleo-cholide radicle. If, on addition of sugar, it gives the purple, then it contains the oleo-cholide radicle; if it does not give the purple, then this radicle also is excluded.

I will now proceed to the application of these data to the testing of the chemolytic products above described. Sphingosin, with oil of vitriol at a very gentle heat, dissolves and becomes a little yellow. But no purple colour is produced. On addition of cane-sugar or of cerebrose in highly concentrated solution the purple is immediately struck. Psychosin, with oil of vitriol at a very gentle heat, becomes yellow and brownish while dissolving, and then the purple colour appears without any addition of sugar. Consequently there is a *primâ facie* presumption that psychosin still contains the cerebrose, while from sphingosin it is detached. We have already seen how well this presumption, derived from the chemical reactions of these bodies, is supported by their relative chemical formulæ. The reaction can consequently be used for demonstrating the purity or impurity of any specimen of sphingosin as regards its freedom from or contamination with substances capable of yielding cerebrose with oil of vitriol. The substances most likely to remain mixed with sphingosin in

small quantity are those of which it is a cleavage-product, and more particularly psychosin, which, like sphingosin, is an alkaloid.

Isolation of the Purple Products.—The purple bodies produced in the reaction of the cerebrin-products described in the foregoing pages are, under certain conditions, soluble in chloroform ; it is necessary to place the mixture in bottles carefully stoppered, and keep them anhydrous by excess of oil of vitriol. The clear dry chloroform solution can be distilled from dry vessels boiling, and leaves the purple product behind. The residue dissolves in new chloroform with a finer purple colour than before and completely. A little water added to the purple chloroform solution makes it turbid, and destroys the colour completely in a few minutes. The purple, which has been redissolved after the removal of the first chloroform by distillation, is destroyed by water instantaneously.

Caramel of Psychosin.—*Experiment.*—0·3615 g. heated at 110° C., in the apparatus used in the other cases, lost ·021 g., and the $CaCl_2$ gained ·0225 g. At this temperature the substance became brown and caked. It was then heated to 160° C., when it fused completely, losing ·0145 g., the gain being at the same time ·0185 g. At 210° C. it had lost ·021 g., while the $CaCl_2$ had gained the same amount. On cooling, it split up into flakes, which were only partially soluble in ether. At 210° C. the current of air was stopped, as there was a slight deposit of volatile matter on the cooler parts of the tube. The current was resumed as the caramel cooled.

Tabular View of the Data concerning the Caramel of Psychosin.

Dry Amount.	Temp.	Per cent. Loss.	Total per cent. Loss.	Molecles.	Per cent. Gain.	Total per cent. Gain.	Molecles.
·3615	110°	5·81	—	—	6·22	—	—
—	160°	4·01	—	—	5·12	—	—
—	210°	5·81	15·63	4·038	5·81	17·15	4·43

Remarks on the Caramels.—The preliminary experiment on the action of heat upon phrenosin showed a loss of 10·2 per cent., which is equal to 4·03 molecles of water on the formula $C_{41}H_{79}NO_8$. The maximum loss which phrenosin experiences in the experiments described later is similar in amount, but the

water collected does not tally with it. It is therefore clear that some oxidation takes place in the substance under caramelisation; this hypothesis does not, however, completely explain the discrepancies. In future experiments the operation should be carried on in a current of neutral gas, such as hydrogen or nitrogen, or in carbonic acid.

f. Intermediate Products of the Chemolysis of the Cerebrosides with Sulphuric Acid: Hydrated Phrenosin, Æsthesin, Psychosin.

The decomposition of the cerebrosides by barita ensues in a much shorter time than by acids. The products, though essentially the same in both processes, are differently distributed, and are split off at different times and in a different order. It is essential to know all those products which are intermediate, in the first instance, because without them the formulæ of the decomposition cannot be made evident with all necessary detail, and secondly, because small quantities of these intermediate products mostly outlast the chemolytic process, and then occur as impurities in the final products or are left as residues incapable of purification and analysis.

The first event in the sulphuric acid chemolysis of phrenosin is probably *hydration*. The next event is the *splitting off of the cerebrose.* This, in the water solution, ensues very slowly, while the first hydration is probably more quickly attained.

The remainder of the radicles, minus the cerebrose, do yet hold together for a longer time before they split up into sphingosin, fatty acids, and other bodies.

Forty-four g. of mixed cerebrosides were boiled in water, and pressed through a cloth. To the homogeneous paste 40 cc. of oil of vitriol, already diluted with the amount of water necessary to prevent overheating, were added. The mixture was now boiled, and curdled immediately. Boiling was continued for nearly an hour, when, the liquid containing much sugar and becoming more coloured, heat was withdrawn.

The *curdled cerebrin matter* was transparent and soft while hot, slightly coloured red, and became solid and white immediately on cooling. In order to remove any fatty acids which it might contain, it was, after removal of all sulphuric acid, suspended in ammonia water, and precipitated by barium chloride. The curdy

precipitate was found to be almost entirely soluble in hot spirit, and to contain hardly any fatty salt insoluble in spirit.

The spirit solution filtered hot through a heated funnel immediately deposited *white crystals.* These, under the microscope, were seen to consist of two bodies, one in needles, another in crystalline balls. The deposit from spirit was isolated and suspended in ether.

A *body dissolved* which crystallised from the evaporating ether in apparently curved needles, which will presently be more closely defined. Another body remained insoluble in ether, and on combustion left some barita. This latter will not be considered any further in this place.

Crystallised soluble in Ether Product.—Æsthesin.—The ether solution was distilled to a small bulk, and then allowed to crystallise. It formed a voluminous white mass consisting entirely of crystals, which were uniform and showed many angular plates. They were collected on a filter and drained of mother-liquor by stirring. While wet they fused on the water-bath in the mother-liquor adhering to them, but became completely dry without much discoloration, and were solid and waxy, therefore not fusible below 90°. Recrystallised slowly from a dilute solution in ether, the crystals are seen to be hexagonal plates, more or less regular, but whether or not the six angles of the hexagons are equal cannot be determined. The plates are saucer-shaped, and this produces the appearance of the curved or sickle-shaped needles when the bodies are seen sideways and the lower edge is out of focus. The plates are scarcely visible when they lie flat on the glass. They are distinctly recognised with all the details when they are made to roll edgeways over the field (Chinese hats).

The dry crystals dissolve in oil of vitriol and assume a yellowish colour, but no purple colour. Cane-sugar added to this produces the purple reaction soluble in chloroform and yielding a specific spectrum; consequently, this body contains the oleocholide radicle, but not the cerebrose radicle. It was now analysed. Between 80° and 150° 0·1825 g. lost in three stages 5 mgrs. in weight; the remainder burnt left 1 mgr. incombustible residue. The dry powder fritted a little, and at 82° to 83° became a waxy transparent mass. It fused to a liquid at 110°, but did not become opaque until again cooled to 71°. By recrystallisation

its nitrogen, which at first was 2·70 per cent., could be depressed to 2·35. After purification and crystallisation from absolute alcohol and from pure ether, in both of which it was soluble in the cold, it crystallised in the same hexagonal plates. Heated to 150° it became coloured, and exhaled an odour of burnt fat. For analysis it was heated to 100° for three hours, and gave results which are represented in the following summary :

	Percents.	÷At. Wgts.	÷N=1.
C	74·83	6·24	37·14
H	12·73	12·73	75·79
N	2·35	·168	1·00
O	10·09	·631	3·75

These results prove that æsthesin is an intermediate product of chemolysis ; it does not contain the cerebrose radicle, as is evident from the low quantity of oxigen it contains, and it still retains the oleo-cholide radicle, as is evident from its yielding the purple reaction when sugar is added. It therefore behaves like a sphingosin to which a fatty acid radicle is still combined. The nitrogen is a little too low; that is to say, æsthesin is already mixed with a trace of fatty acid, but the oxigen stands to carbon in the relation of about 3 atoms to 35 atoms. Hence the hypothesis that æsthesin is a compound of sphingosin and neurostearic acid gains a high degree of probability, thus :

$$\overbrace{C_{17}H_{35}NO_2}^{\text{Sphingosin.}} + \overbrace{C_{18}H_{36}O_2}^{\text{Neurostearic acid.}} = \overbrace{C_{35}H_{69}NO_3}^{\text{Æsthesin.}} + \overbrace{H_2O}^{\text{Water.}}.$$

If now to æsthesin we add a molecle of cerebrose, we obtain—

$$\overbrace{C_{35}H_{69}NO_3}^{\text{Æsthesin.}} + \overbrace{C_6H_{12}O_6}^{\text{Cerebrose.}} = \overbrace{C_{41}H_{79}NO_8}^{\text{Phrenosin.}} + \overbrace{H_2O}^{\text{Water.}}.$$

The fatty acid radicle contained in æsthesin is supposed to be neurostearic acid.

Theory.			Found.	Theory of a Hydrate.		
Atoms.		Percents.	Percents.	Atoms.		Percents.
35 C	420	76·22	74·83	35 C	420	73·81
69 H	69	12·52	12·73	71 H	71	12·47
1 N	14	2·72	2·35	1 N	14	2·46
3 O	48	8·54	10·09	4 O	64	11·26
	551	100·00			569	

The æsthesin analysed did, therefore, yet contain about half a molecle of water. The body seems to be a base.

g. Chemolysis of Phrenosin by Sulphuric Acid in Alcoholic Solution; Formation of Ethylic Neurostearate.

The Process.—Sixty-five g. phrenosin were suspended in 1500 cc. of spirit of 85 per cent. strength, and 200 cc. of oil of vitriol gradually added while the mixture was well agitated. The hot mixture, on which an oily matter floated, was then boiled for two hours and a half in such a manner that the volatilised alcohol was condensed and ran back again into the fluid. On cooling, the oily layer solidified, and a slight deposit formed in the underlying fluid. The insoluble and deposited parts were separated by filtration from the acid solution and dissolved in ether; the ethereal solution was agitated with dilute solution of caustic soda, by which a small quantity of fatty acid was removed as soda salt. The clarified ether solution was distilled, and the residue was repeatedly crystallised from absolute alcohol. From this solvent it was deposited in small shining crystals, which were very soluble in hot alcohol, but very little in cold. The recrystallised matter fused at 56° C., but the manner of its fusion seemed to indicate an admixture of a less fusible with a more fusible body. It was subjected to a preliminary elementary analysis by the vacuum method with the following result: 0·0872 g. gave 0·1080 g. H_2O, 0·2443 g. CO_2, and 0·00043 g. nitrogen. The small amount of nitrogen was evidently due to the presence of a small quantity of a nitrogenised substance which was at once eliminated by the process of distillation in vacuo about to be described. The recrystallised body was mixed with glass powder and placed in a glass tube closed at one end. This was then bent downwards at a point beyond the mixture, and a little further on bent again upwards, drawn out and connected air-tight to a mercurial air-pump. The air was then completely pumped out, and heat cautiously applied until the matter was distilled into the V-shaped part of the tube; this was effected without the evolution of any gas and without a trace of charring. When at the end of the distillation the slight residue in the glass powder began to discolour and to evolve gas, the operation was stopped.

Neurostearic Ether, $C_{20}H_{40}O_2$.—The distilled matter was of the colour and consistence of bleached beeswax. It melted at 52° C.

On analysis it gave numbers agreeing with those demanded by the formula $C_{20}H_{40}O_2$. It contained no trace of nitrogen.

	Theory.		Found.
	Atoms.	Percents.	
C_{20}	240	76·93	76·69
H_{40}	40	12·82	12·95
O_2	32	10·26	10·36
	312	100·00	100·00

The body is ethylic neurostearate $(C_2H_5)C_{18}H_{35}O_2$, as was proved by the following experiment: 4·5 g. of the ether, which had been completely freed from any adhering alcohol by fusion at 110° during several days, were enclosed in the platinum chemolyser with concentrated soda ley, and heated to 100° for eight hours, during which the chemolyser was frequently shaken. The caustic solution when quite cold was poured upon a filter of glass wool, and by this means the soap, which was quite insoluble in the concentrated ley, was separated from the excess of alkali; and, after rinsing with cold, was dissolved in a large quantity of boiling water. It gave a clear solution. A portion of this solution, precipitated hot with barium chloride, gave a barium salt. This was converted into free acid, which was dried and dissolved in ether. On concentration, the ether gave a crystallisation of perfectly white neurostearic acid in a characteristic form. The ethereal mother-liquor crystallised similarly in cauliflower masses to the last drop. This acid fused at 84° C. It was dried by fusion at 100° C., and analysed with the following results:

Theory.			Found.
Atoms.	At. Wgts.	Percents.	Percents.
C_{18}	216	76·06	75·94
H_{36}	36	12·68	12·64
O_2	32	11·26	11·42
	284	100·00	100·00

The concentrated soda ley employed in this chemolysis of the neurostearic ether was examined for alcohol. It was distilled, the distillate enclosed hermetically in a glass tube with excess of chromic and sulphuric acids, and heated for six hours to 100°. The chromic acid was partially reduced, and on distillation of the fluid an acid distillate was obtained. This, after boiling with

barium carbonate, and evaporation to dryness, gave 0·32 g. dry barium acetate, giving a red coloration with ferric chloride, evolving the pungent fumes of acetic acid when moistened with oil of vitriol, and giving the fragrant odour of acetic ether with alcohol and oil of vitriol.

Psychosin Sulphate.—The alcoholic mixture of sulphuric acid and other decomposition products from which the neurostearic ether had been removed by filtration, was treated with caustic lime in powder, and the liquid, now free from sulphuric acid, filtered from the gypsum and some calcium neurostearate which was mixed with the precipitate. The alcoholic solution was distilled, and, after the alcohol had passed off, left a voluminous white pasty mass of free psychosin. This was dissolved in hydrochloric acid and ether, and thus separated from the impurities, of which sulphovinate of calcium was the principal one. The ether solution of psychosin hydrochlorate was freed from ether by distillation, the residual salt dissolved in water, and allowed to stand in the cold. It deposited colourless crystalline plates, masses of fine microscopic crystals of extreme tenuity, but showing geometric definition of sides and angles; these easily redissolved if the liquid was allowed to rise in temperature. Filtration was therefore effected in the cold. These crystals consisted of pure sphingosin hydrochlorate, as was proved by isolation, decomposition with caustic potash in the hot, extraction of the base with ether, solution of the base, after distillation of the ether, in absolute alcohol, and precipitation of the sulphate by oil of vitriol dissolved in absolute alcohol. The psychosin in the solution was identified by isolation and all the characteristic tests.

h. Action of Heat upon Phrenosin; Formation of a Caramel.

Preliminary Experiments.—Some preliminary experiments for the study of the action of heat upon phrenosin may be here again referred to (see 'Reports,' etc., New Series, No. III., 1874, p. 191). '0·5290 g. lost, on drying between 18° and 70° C., 0·0084 hygroscopic water, and the remaining 0·5206 g. was considered as dry substance. After two hours' exposure to a heat of 97° C. in an air-bath it became slightly yellow, and had lost 0·0016 g. After three hours' exposure to 101° it had remained of the same colour and weight. After two hours' exposure to 145° it had become

very dark, almost black in colour, and superficially fused, and had lost 0·0080 g. After heating to 158° during three hours it had become thoroughly fused, and of a reddish, almost transparent aspect; it had lost 0·0120 g. in weight. After four hours at 177° C. it had become blacker and less transparent, and gave out a faint odour of burnt meat; it had lost at this stage 0·0317 g. Thus the phrenosin had lost in four stages 0·0533 g., or 10·2 per cent. in weight. After cooling, it was hard and brittle. On boiling a piece in absolute alcohol, only a trifling amount of matter dissolved, colouring the solution slighty yellow. On the other hand, it readily dissolved in ether; the solution had a dark reddish-brown colour. A little of the phrenosin was heated in a test-tube over the naked flame. It fused, turned dark, and evolved water with ebullition, similar to sugar passing into caramel. The water, which condensed in the upper part of the tube, had an acid reaction, and reduced copper solution. The fused matter became hard on cooling, was but slightly soluble in boiling alcohol, but readily soluble in ether; from the latter solution it was reprecipitated by alcohol.'

Similar experiments were now instituted, with a view of increasing the accuracy of results and the knowledge concerning the constitution and function of phrenosin.

First Experiment.—Some phrenosin was dried in a current of air at 100° C., in a Liebig's drying-tube placed in a paraffin bath, connected at each end with a chloride of calcium drying-tube, one to dry the air before it entered the tube, and the other to arrest the moisture from the phrenosin evolved during the operation. A drying-tube filled with glass saturated with sulphuric acid served to prevent moisture from the water-pump diffusing backwards into the chloride of calcium tube.

The dry phrenosin weighed 1·216 g. The temperature was raised to 150° C., a current of dry air passing meanwhile. When the tube containing the phrenosin was weighed, it was found to be still of the same weight as at the beginning of the experiment; but the chloride of calcium tube had gained ·019 g., a fact which can only be explained by oxidation of the phrenosin. At this stage the phrenosin became brownish-white in colour, but did not fuse. The temperature being now raised to 160°, the phrenosin melted into a brown mass, and on being weighed was found to

have lost ·014 g., the chloride of calcium tube having gained the same amount. The temperature was then rapidly raised to 200° C., but there was no longer a current of air allowed to pass through, in order to retard as much as possible the evolution of volatile matter. On cooling, air was passed through as before, and the tube on being weighed showed a loss of 0·054 g., the chloride of calcium tube having only gained 0·050 g. The temperature was now finally increased to 240° C., with the same precautions against escape of volatile matter as before. Volatile matters were now, however, evolved which slightly blackened the sulphuric acid; and when the connections of the apparatus were severed, a smell of burnt sugar was perceived. The tube showed a loss of 0·080 g., while the chloride of calcium tube had gained 0·056 g.

Thus in the whole operation the tube had lost 12·17 per cent., while the chloride of calcium had gained 11·425 per cent. Taking the formula of phrenosin as $C_{41}H_{79}NO_8$, the molecular weight of it would be 713. The preceding figures would then give us, taking the loss as 12·17 per cent., a loss of 4·82 molecles of water; and, taking the gain as 11·425 per cent, a loss of 4·52 molecles.

Second Experiment.—Some phrenosin was dried at 100° C., the weight of the dry body being 0·789 g. The temperature of the tube was now raised to 200° C., and kept at that point for two hours, air being passed through only on cooling below 150°. No volatile matters were carried into the sulphuric acid tube, though a slight sublimation of volatile matter on to the cooler parts of the tube took place. On weighing, the tube was found to have lost 0·0456 g., and the chloride of calcium had gained 0·054 g. The heating was now continued at 200° for four hours, to try the effect of time on the operation, air being passed through only on cooling. The loss was now 0·0365, and the gain 0·0365. The totals in percents. were a loss on the part of phrenosin of 10·14 per cent. or 4·02 molecles, and a gain on the part of the calcium chloride of 11·47 per cent. or 4·54 molecles of water.

The greater portion of the caramel obtained in both these experiments was soluble in ether; the first caramel was not so perfectly soluble as the second, on account of the greater temperature to which the former had been raised.

Tabular View of the Data concerning Caramel of Phrenosin.

Dry Amount.	Temp.	Per cent. Loss.	Total per cent. Loss.	Molecles.	Percent. Gain.	Total per cent. Gain.	Molecles of H_2O.
1·216	150°	·00	—	—	1·56	—	—
—	160	1·15	—	—	1·15	—	—
—	200	4·44	—	—	4·11	—	—
—	240	6·58	12·17	4·82	4·605	11·425	4·52
·789	200	5·89	—	—	6·85	—	—
—	200	4·25	10·14	5·02	4·62	11·47	4·54

i. Special Reactions of Phrenosin.

Reaction of Phrenosin with Oil of Vitriol, Chloroform and Glacial Acetic Acid ; Spectral Phenomena of the Product.—Phrenosin from the brain of man was suspended in chloroform, and sulphuric acid added ; this was allowed to stand five minutes, and the chloroform was then decanted; it remained white and clear. On being allowed to stand over the sulphuric acid all night the chloroform was still colourless, but the sulphuric acid was darker; the latter was dissolved in glacial acetic acid with a little sulphuric acid in order to dissolve some red particles. The solution so produced had a magnificent red colour, and presented spectra as follows: In a concentrated solution red only passed. This solution had a green fluorescence. More diluted three bands appeared, one between D and E, a second between E and F, and a third between F and G.

Reaction of Phrenosin with Pettenkofer's Test; and Spectrum of its Product.—Phrenosin from the brain of man was dissolved in boiling chloroform; it became turbid on cooling, but did not become congealed. This solution was now mixed with sulphuric acid and sugar, then stirred and repeatedly breathed upon; it formed purple oily drops, but no solution took place. These drops were insoluble in glacial acetic acid, but dissolved rapidly in chloroform. The solution, kept anhydrous by sulphuric acid, presented the following spectrum with Drummond's light: a narrow band between C and D, and a wide deep black band between D and *b*. These reactions of phrenosin are very similar to those of kerasin to be described. They are also very similar to those of oleic acid.

k. Theory of the Chemical Constitution of Phrenosin.

Although the average of the analyses of phrenosin leads to data from which the true formula of its composition can be derived, it is a great advantage to be able to prove the true formula by the aid of the formulæ of the products of decomposition. If phrenosin had never been analysed by itself, its formula could be predicted by means of the synthesis of its cleavage-products, according to the following calculation:

1 molecle of sphingosin	-	$C_{17}H_{35}NO_2$.
+1 ,, of neurostearic acid	-	$C_{18}H_{36}\ O_2$.
+1 ,, of cerebrose	-	$C_6\ H_{12}\ O_6$.
− 2 molecles of water	-	$H_4\ O_2$.
leave one molecle of phrenosin	-	$C_{41}H_{79}NO_8$.

Arranged analytically the equation would be the following:

$$C_{41}H_{79}NO_8 + 2(H_2O) = C_{17}H_{35}NO_2 + C_{18}H_{36}O_2 + C_6H_{12}O_6.$$

Of this phrenosin the atomic and percentic theory is the following:

Theory.			Found mean.
Atoms.		Percents.	Percents.
41 C	492	69·000	67·957
79 H	79	11·080	11·426
1 N	14	1·963	1·997
8 O	128	17·957	18·696
	713	100·000	

This phrenosin, in the course of chemolysis, by taking up two molecles of water, increases its atomic weight from 713 to 749, which latter figure represents the sum of the atomic weights of the three products of decomposition above enumerated. 100 parts of phrenosin, therefore, become 105 parts of products. Of these 39·6 parts are neurostearic acid, 39·9 parts sphingosin, and 25·1 parts cerebrose.

If now, with the aid of this theory, we attempt to revise the theories formerly given of some compounds of phrenosin, retaining the analytical data, we obtain a greater harmony than before. Thus the nitrited phrenosin shows the following theory:

Nitrited Phrenosin Nitrate, $C_{41}H_{78}(NO_2)NO_8 + HNO_3$, or contracted, $C_{41}H_{79}N_3O_{13}$.

Theory.			Found in 100.
Atoms.		Percents.	
41 C	492	59·92	59·58
79 H	79	9·62	9·93
3 N	42	5·11	4·65
13 O	208	25·33	25·84
	821		

Neurostearic Acid is accurately distinguished from the isomeric known common stearic acid by its high fusing-point (84° to 85°), and its atomic weight is well fixed by its ethyl compound. But its salts are not very stable nor very precise bodies, or are mixtures, so that it has hitherto proved impossible to use them for stoichiometric purposes. The theory of this acid is the following:

Theory.			Found	
Atoms.		Percents.	in Crystallised Acid.	in Acid from Ether.
18 C	216	76·056	75·88	75·94
36 H	36	12·676	12·85	12·64
2 O	32	11·268	11·27	11·42
	284	100·000	100·00	100·00

The ethylic compound of this acid is a very precise body; it is *Neurostearic Ether* or ethylic neurostearate, and its theory is the following:

Theory.			Found.
Atoms.		Percents.	Percents.
20 C	240	76·92	76·69
40 H	40	12·82	12·95
2 O	32	10·26	10·36
	312	100·00	100·00

The dissolved formula is $(C_2H_5)C_{18}H_{35}O_2$.

Sphingosin is a strong base, and gives the most precise compounds of any products of the decomposition of phrenosin. Its theory is the following:

Theory.			Percents. found in Sulphate, Acid deducted.
Atoms.		Percents.	
17 C	204	71·59	71·26
35 H	35	12·28	12·53
1 N	14	4·91	4·82
2 O	32	11·22	11·39
	285	100·00	100·00

Sphingosin Sulphate, $2(C_{17}H_{35}NO_2) + H_2SO_4$.

Theory.				Found.
Atoms.			Percents.	Percents.
34	C	408	60·11	60·85
72	H	72	10·78	10·70
2	N	28	4·19	4·14
4	O	64	10·55	9·99
1	S	32	14·37	14·32
4	O	64		
		668	100·00	100·00

Cerebrose, $C_6H_{12}O_6$, is a sugar characterised by its crystallisation, its optical power (its specific or limited rotation being + 70° 40′), and its reducing power over cupro-potassic tartrate. Its theory is the following :

Theory.				Found.
Atoms.			Percents.	Percents.
6	C	72	40·000	39·93
12	H	12	6·666	6·71
6	O	96	53·334	53·36
		180	100·000	100·00

Under certain conditions which have been described above, cerebrose is changed into an acid isomeric with cerebrose, and therefore of the formula $C_6H_{12}O_6$. This *cerebrosic acid* is dibasic, *i.e.*, contains two atoms of hydrogen replaceable by metals. The theory of the *cerebrosate of barium* is the following :

Theory.				Found.
Atoms.			Percents.	Percents.
6	C	72	22·85	24·53
10	H	10	3·17	3·2
1	Ba	137	43·49	43·5
6	O	96	30·49	
		315		

This acid is remarkable in this, that it has not got any reducing power over potassio-cupric tartrate, but on the other hand gives with an oleo-cholide radicle, *e.g.* sphingosin, and oil of vitriol the purple reaction in the same manner as cerebrose.

Psychosin, $C_{23}H_{45}NO_7$, is the cerebroside of sphingosin ; it is crystallisable from alcohol ; it is an alkaloid, but of less pro-

nounced character than sphingosin ; it forms salts with acids, which are more or less soluble in water, the hydrochlorate being very soluble indeed. By this solubility in cold water it can be separated almost completely from the hydrochlorate of sphingosin, which crystallises from cold water, or cold solution of psychosin hydrochlorate.

Theory				Found. Percents.	
Atoms.			Percents.	*a.*	*b.*
23	C	276	61·74	61·86	61·32
45	H	45	10·06	10·09	10·09
1	N	14	3·13	2·88	3·04
7	O	112	25·07	25·17	25·55
		447	100·00		

One hundred parts of psychosin on chemolysis should take up 4·02 parts of water (one molecle), and then split up into 40·29 parts of cerebrose and 63·75 parts of sphingosin.

The hydrochlorate is completely precipitated by excess of hydrochloric acid. Many compounds may be expected to be obtained, as psychosin exhibits numerous promising reactions. It gives the oleo-cholide reaction with oil of vitriol alone, showing that it contains the cerebrose and sphingosin radicles.

The *Caramels* of the cerebrosides are, like the caramels of the sugars, produced by the expulsion of water under the influence of heat. They are all soluble in ether, insoluble in alcohol and in water, and of a deep brown colour. The following formulæ are hypothetical and interimistic, although derived from the data of the experiments given above.

Caramel of Phrenosin, $C_{41}H_{71}NO_4$, formed from a molecle of phrenosin, $C_{41}H_{79}NO_8$, by the loss of four molecles of H_2O.

Caramel of Psychosin, $C_{23}H_{37}NO_3$, formed from a molecle of psychosin, $C_{23}H_{45}NO_7$, by the loss of four molecles of water.

A small quantity of caramel is formed during every chemolysis of any of the cerebroside principles, with acid or with alkali, even during the chemolysis of psychosin with dilute sulphuric acid. There may be several varieties of caramel of each principle produced by the loss of one, two, three, or four molecles of water. There might be mixed with some preparations of phrenosin particular varieties of phrenosin, in very small quantity, containing in place of the neurostearic another fatty acid radicle. And there

might be mixed with an hypothetical di-neurostearyl-sphingosyl-cerebroside, a similarly constituted body containing in place of one or both molecles of neurostearyl other fatty acid radicles, and in place of the sphingosyl another nitrogenised radicle ; in this respect the nitrogenised principles might imitate the phosphorised principles, which derive their principal differences from the different fatty acid radicles which they contain. I think it even very probable that some of the phosphorised bodies which cling so pertinaciously to the nitrogenised ones owe their similarity to these latter, both in shape and chemical properties, to the fact of their containing one or other or both of the radicles of the nitrogenised bodies which appear as neurostearic acid and sphingosin.

3. Kerasin, the Second Cerebroside : its Isolation and Properties.

Introduction.—As in the case of phrenosin, I have, up to the present moment, not succeeded in isolating a preparation of kerasin which, on analysis of a sufficient quantity, proved to be free from phosphorus. The preparation which yielded the lowest amount of phosphorus, namely, 0·08 per cent. (8 parts of phosphorus in 10,000 parts of kerasin), weighed only 4 g., so that after six quantations, for which the materials were taken from these 4 g., there was not left material enough to be employed in a systematic attempt at chemolysis of this particular specimen. But I have examined a considerable number of preparations of kerasin which contained more of the phosphorised impurity, not exceeding 0·4 per cent. of P ; and some of these specimens have yielded information which, when compared with the information derived from the purest specimens, seemed to be independent of the influence of the thus far unavoidable impurity. But the information, though decisive as far as it goes, is only fragmentary as regards the entire problem ; and I therefore point out that what I have to report is only the beginning of a great research to be made in the future.

Mode of Isolation.—The ox white matter, which had been extracted with ether and dried, was pulverised and dissolved in hot absolute alcohol ; the solution was decanted from the fused mass of stearoconot which formed, and allowed to deposit the dissolved matter by cooling. The deposit formed after the first hour was isolated, as was also another which formed after the second hour ;

a third gelatinous-looking precipitate formed over-night, and from this the absolute alcohol solution was filtered. On standing for a few days in stoppered bottles, this solution deposited a gelatinous membranous mass, mainly consisting of *kerasin.* This was removed by the filter, and the filtrate, which contained much *sphingomyelin* in solution, was treated with $CdCl_2$. The white bulky precipitate of sphingomyelin $CdCl_2$ was filtered off, washed, exhausted with ether, and further treated as is described elsewhere. It may be mentioned here that the principal bulk of sphingomyelin is obtained in this manner and at this stage. The alcohol filtered from the sphingomyelin $CdCl_2$ precipitate after concentration deposited a mixture of $CdCl_2$ salt, cholesterin, and kerasin, the latter two being present in very small proportions.

Mode of Purification.—Kerasin obtained as above is a soft white gelatinous mass, consisting of larger and smaller balls, which under the microscope are seen uniformly to consist of wavy masses of needles so thin that it may be said they possess only one diameter—namely, length. The gelatinous state is apparently entirely due to this peculiarity of the fine needles enclosing a large amount of alcohol. No amorphous matter whatever is seen mixed with it, but here and there a few rosettes of phrenosin, strikingly differentiated from the kerasin.

The whole of the kerasin was dissolved three times in boiling absolute alcohol, and, after cooling to crystallisation, washed and pressed free from mother-liquor. This, after the third operation, was free from sphingomyelin, as shown by the absence of reaction with $PtCl_4$, and $CdCl_2$. The solution of kerasin was also free from sphingomyelin, $CdCl_2$ giving no precipitate with it.

The kerasin was now dissolved in a fourth quantity of pure absolute alcohol, and allowed to crystallise. It was found by microscopic examination that after three hours much kerasin in wavy crystallised masses, but no phrenosin in rosettes, had been deposited. The crystals were consequently isolated, pressed, again recrystallised, collected on a filter, and dried in vacuo. This preparation was analysed with the result stated below.

After this preparation had been removed, at the end of the third hour, from the mother-liquor, the latter on standing deposited a mixture of much kerasin in wavy needles, with some rosettes of phrenosin. This mixture could by recrystallisation

not be completely separated. A third ultimate precipitate seemed to consist mainly of phrenosin, with much kerasin, which could also not be purified by mere recrystallisation.

Special Consideration of the Properties of Kerasin which are made use of for its Isolation.—The properties of kerasin which are made use of for its isolation are the following: It is easily soluble in hot spirit, and almost insoluble in cold; it tarries to deposit from this solution for a long time, and if its amount does not exceed 1 part in 321 parts of spirit, it is not deposited at all above 28°, and below that temperature very slowly. By this peculiarity it is separated from phrenosin and sphingomyelin, the latter particularly when it is in the state of cadmium chloride salt. Kerasin does not combine with lead; by this property it is separated from myelin and the cerebrinacides, which combine with lead. It does not combine with cadmium chloride, and by this reagent is liberated from sphingomyelin, amidomyelin, and paramyelin, which combine with it. It is not soluble in ether, either cold or boiling; by cold ether the kephalins and lecithins are extracted from it, and by boiling ether, krinosin. Kerasin swells when left in contact with ether for some time. In that state it must not be allowed to dry on paper, as it contracts and becomes hard, and adheres so strongly to the paper that it cannot be separated from it without retaining fragments of paper in its substance. Kerasin, crystallised from absolute alcohol in a flask, if allowed to dry slowly in the flask after the alcohol has been poured off, becomes white and pulverulent, so as to crumble off the sides of the glass, and does hardly become waxy. When deposited from watery solvents, or removed from contact with even anhydrous ether, it becomes on drying hard and horny, or waxy, and difficult to powder.

Solubility of Kerasin in Different Quantities of Spirit.—3·8878 g. human kerasin, dried on the water-bath, were dissolved in 500 cc. hot spirit of 84 per cent. strength. On cooling of the solution a deposit of kerasin began to form at 40°, and continued to increase while the temperature sank. 200 cc. spirit were added to the mixture, and the deposit was redissolved by the application of heat. On being again allowed to become cool, it now became turbid, and began to make a deposit at 35°. A further addition of 50 cc. of spirit reduced the depositing-point to 34°. Another 100 cc. reduced the crystallising-point to 33°. A further 100 cc.

depressed the temperature of crystallisation to 32°. Three more additions of 100 cc. spirit each depressed the crystallising-point to 30°, 29°, and 28° respectively. Altogether, therefore, 3·8878 g. kerasin required 1250 cc. of spirit to be kept in solution at 28°; one part of kerasin required 321 parts of spirit to remain in solution at 28°.

Fifty cc. of a solution of kerasin in spirit, which began to crystallise at 28°, were at that temperature filtered and evaporated to dryness on the water-bath. The residue weighed 0·1558 g.; therefore 1 g. kerasin required 320·92 cc. of spirit at 28° for solution.

Solubility of Kerasin in Aceton.—At the ordinary temperature of the air, 100 cc. of aceton dissolve 0·1576 g. kerasin; at the boiling temperature of aceton, 100 cc. dissolve 1·0510 g. kerasin.

Solubility of Kerasin in Benzol.—When moist kerasin is shaken with large volumes of benzol, it remains as an insoluble gelatinous transparent mass. The filtered benzol leaves no residue on distillation. When kerasin and benzol are warmed, a perfect solution is produced, which can be filtered hot. On cooling, all kerasin is deposited, and the benzol leaves no residue on distillation. This solvent, therefore, may serve for the separation of bodies which are soluble in cold benzol.

Reactions of Kerasin.—Both human and bovine kerasin give a deep purple with oil of vitriol and sugar at once; with oil of vitriol alone, a paler purple after long standing. Kerasin from ox-brains is dissolved in boiling chloroform. On cooling, it congeals to a glassy solid. A portion of this is stirred with a drop of cane-sugar solution and a small quantity of sulphuric acid. At first it becomes yellowish, and at last purple. The colour is in drops, and not dissolved; it is not soluble in glacial acetic acid, but chloroform dissolves the whole to a purple solution. This before the spectroscope shows a narrow band between C and D, and a deep black band extending from D to F. The acid solution below the chloroform is yellowish, fluorescing green. When some kerasin is dissolved in chloroform, and sulphuric acid added, all the kerasin passes into the acid, and the chloroform remains colourless; this proves that the kerasin is free from cholesterin. When this mixture is allowed to stand, a brownish-red mass rises to the top of the acid; sugar added to this makes it redder. When this is placed in a dish and the chloroform

evaporated, oily deposits form of increased redness, the oxigen of the air evidently tending to make them purple; but this purple product is now insoluble both in acetic acid and chloroform, singly or united.

Kerasin, in a Liebig's drying-tube, heated in an oil-bath to from 100° to 150° in a current of dry air, loses about 4 molecles of water, and is transformed into a brown matter soluble in ether, insoluble in alcohol (caramel of kerasin).

Elementary Analyses and Theory of Kerasin.—A specimen of kerasin from the ox obtained from 70 g. of the principle by frequent fractional recrystallisation, and containing yet 0·01 per cent. P, was analysed, and yielded the first group of data in the synopsis below.

A specimen of human kerasin was repeatedly crystallised from absolute alcohol, allowed to dry, and crumble in flask, and dried over oil of vitriol in vacuo. It contained 0·073 per cent. P; it gave on analysis the second set of data in the synopsis.

If we group these analyses so as to let the highest nitrogen come first, we obtain the following hypotheses:

Synopsis of Analyses of Kerasin from Ox.

	Percents.	÷ At. Wgt.	÷ N=1.
C	69·54	5·795	42·29
H	11·69	11·690	85·32
N	1·92	0·137	1·00
O	16·85	1·053	7·68
	100·00		

Synopsis of Analyses of Kerasin from Man.

	Percents.	÷ At. Wgt.	÷ N=1.
C	69·01	5·75	44·23
H	11·44	11·44	88·00
N	1·90	0·13	1·00
O	17·65	1·10	8·46
	100·00		

Synopsis of Analyses of Kerasin from Ox, made in 1874.

	Percents.	÷ At. Wgt.	÷ N = 1.
C	68·446	5·704	46·0
H	11·395	11·395	92·0
N	1·738	0·124	1·0
O	18·421	1·151	9·2

The two first sets of analyses are probably to be preferred, and it is very probable that the formula of kerasin is $C_{42}H_{85}NO_8$, or $C_{44}H_{89}NO_8$; any formula giving oxygen as eight atoms has theory in its favour, as we shall see below. But in any case the formula cannot be established by analysis alone; chemolyses of kerasin and analyses of the cleavage products are required to enable us to form a final opinion as regards its chemical constitution.

Chemolysis of Kerasin.—Kerasin is a cerebroside, and yields cerebrose by chemolysis with dilute sulphuric acid. In some chemolyses with barita, the study of the acid products of the chemolysis could not be accomplished for want of quantity. But the basic parts were identified in two forms, and the presence of cerebrose was incidentally confirmed.

Four g. of kerasin were mixed with 8 g. of barita hydrate crystals, and sufficient water to produce a thin paste. The mixture was enclosed in a tube and heated during fourteen hours to 100°. The watery solution, free from barita, gave a reaction for cerebrose. The insoluble in water part was extracted with alcohol; the concentrated solution yielded a precipitate of a sulphate. Another part of the matter was not precipitated by sulphuric acid, but remained in solution. It was shown to be psychosin, like the base of the precipitated sulphate. This sulphate dried to a horny mass on the filter, and did not remain pulverulent like sphingosin sulphate. It was dissolved in boiling water, with which it formed a clear solution, and precipitated with a large excess of caustic alkali. It did not rise to the top like an oil, as sphingosin does, on heat being applied, but formed a jelly or semi-soap; with more potash it became emulged; the solution became turbid on cooling; ether extracted nothing from it. The only way to extract the alkaloid was to acidify the solution with sulphuric acid, and add phosphomolybdic acid to it. The precipitate, after decomposition with barita, yielded to spirit the psychosin, which was transformed into sulphate and analysed.

Analyses of the Sulphate obtained, and Comparison with the Composition of Psychosin Sulphate and Sphingosin Sulphate.

	Found in Product from Kerasin.	Psychosin Sulphate. $2(C_{23}H_{45}NO_7)H_2SO_4$.	Sphingosin Sulphate. $2(C_{17}H_{35}NO_2)H_2SO_4$.
C	54·67	55·65	60·11
H	10·48	9·27	10·78
SO_4	10·59	9·67	14·37

As the sulphate yields Raspail's reaction without sugar being added, it must be psychosin, mixed, however, with a small trace of sphingosin.

It follows from the foregoing that kerasin, like its principal companion phrenosin, is a cerebroside, namely, a body which contains the sugar *cerebrose*, combined with at least two other radicles. Of these, one is probably *sphingosin*, the alkaloid obtained from phrenosin. The other is certainly a *fatty acid*, but the nature and composition of this acid have not yet been perfectly ascertained.

B. SUBGROUP OF THE CEREBRINACIDES, OR CEREBRIN BODIES WHICH COMBINE WITH METALLIC OXIDES.

1. General Observations on the Subgroup.

The following account of preliminary observations may serve as the basis for future more perfect inquiries. Of some bodies the mere existence has been ascertained, and they have not been definitively isolated. Others have been isolated in a state of artificial combination with reagents. Two have been isolated in such a state of semi-crystallisation (spherocrystals and groups of microscopic needles) that their appearance and behaviour give some probability to the assumption that they are approximately pure. But the only final control of actual composition, quantation of atomic weight by combination and demonstration of constitution by chemolysis could not yet be applied to them.

Separation of these Substances from the Cerebrin Mixture by Lead Acetate and Ammonia.—This process has already been described, but may here again be noticed. The cerebin mixture, exhausted with ether, is dissolved in hot spirit, and to the solution a hot solution in spirit of acetate of lead is added as long as a precipitate is produced. Ammonia is then added, and when neither lead acetate nor ammonia produce any further precipitate, the solution is filtered hot from the precipitate. The latter contains the bodies here to be considered, while the spirit solution contains the cerebrosides and other substances which do not combine with lead under these circumstances.

The Lead Salts.—The insoluble precipitate formed during the purification of phrenosin by the lead acetate treatment is exhausted with hot 85 per cent. spirit to remove all phrenosin and

kerasin. It is then dried, powdered, and extracted with cold benzol. A portion of the lead salts dissolved, another remained insoluble.

2. Cerebrinic Acid: its Isolation and Properties.

The benzol extracts were evaporated to dryness, and the dry residue was powdered and suspended in 85 per cent. spirit. The spirit was saturated with hydrothion while being gradually raised to the boiling-point, and the lead sulphide filtered off. On cooling a white body was deposited, which consisted mainly of *cerebrinic acid.* This was collected on a filter, purified by recrystallisation from absolute alcohol, and dried in vacuo. It gave a weak purple reaction with oil of vitriol alone. Under the microscope it appeared to consist of small needles. It was soluble in hot benzol, and was thrown down on cooling as a gelatinous mass. It did not blacken at 100° C. On elementary analysis it gave data which are collated in the following table:

	a.	*b.*	*c.*	*d.*
Nitrogen per cent.	1·5	—	—	1·68
Carbon, per cent.	—	66·47	67·54	—
Hydrogen, per cent.	—	11·355	11·37	—

Caramel of Cerebrinic Acid—First Experiment.—1·613 g. of dry cerebrinic acid were heated to 210° C., in a current of air for one hour. The tube lost 0·122 g., or 7·55 per cent.; and the chloride of calcium gained 0·103 g., or 6·38 per cent. Calculated on a formula (hypothetical) of $C_{59}H_{113}NO_9$, and a molecular weight of 979, these figures would give a loss of 4·1 molecles of water, as judged by the diminution of the weight of the cerebrinic acid, and they would correspond to 3·47 molecles of H_2O if only the gain of weight of the water-catching tube were taken into consideration.

Second Experiment.—1·221 g. of dry cerebrinic acid was heated to 160° C. The substance melted completely, and lost 0·030 g., the chloride of calcium gaining 0·033 g. The temperature was now raised to 200° C., and the current of air was passed only when the tube cooled. The loss and gain respectively were 0·065 g. and 0·056 g. The total loss was thus 7·77 per cent., or 4·227 molecles; and the gain was 7·30 per cent., or 3·97 molecles of water.

Third Experiment.—1·073 g. at 150° C. lost 0·012 g., and the chloride of calcium tube gained 0·019 g. At this temperature the cerebrinic acid melted. At 200° C. it lost 0·0965 g., and the chloride of calcium gained 0·080 g. The total percentages were 10·12 per cent. loss, or 5·5 molecles, and 9·23 per cent. gain, or 5·00 molecles of water.

Fourth Experiment.—0·779 g. of dry cerebrinic acid at 145° lost 0·006 g., and the chloride of calcium gained 0·0125 g. At 180° the substance was completely melted, and lost 0·033 g., while the chloride of calcium gained 0·037 g. At 210°, the current of air being stopped as before until the tube had somewhat cooled down, the loss was 0·0535 g., and the gain 0·030 g. The totals lead to a loss of 11·67 per cent., or 6·347 molecles of water, and to a gain of 9·98 per cent., or 5·43 molecles. Oxidation may, as in the former case, account for this discrepancy.

These caramels also were almost entirely soluble in ether.

Tabular View of the Data concerning the Caramels of Cerebrinic Acid.

Dry Amount.	Temp.	Per cent. Loss.	Total per cent. Loss.	Molecles.	Per cent. Gain.	Total per cent. Gain.	Molecles of H_2O.
1·613	210°	7·55	7·55	4·10	6.38	6·38	3·47
1·221	160	2·46	—	—	2·70	—	—
—	200	5·32	7·77	4·227	4·60	7·30	3·97
1·073	150	1·12	—	—	1·77	—	—
—	200	2·66	—	—	1·86	—	—
—	200	6·34	10·12	5·504	5·60	9·23	5·02
·779	145	·77	—	—	1·60	—	—
—	180	4·24	—	—	4·75	—	—
—	210	6·66	11·67	6·347	3·63	9·98	5·43

3. Sulphurised Principles, Sphero-cerebrin, and others.

Mode of separating these Substances from the Cerebrin Mixture.—The cerebrin mixture (that part of the white matter which remains insoluble when it is exhausted with ether) is dissolved in hot spirit, and to the solution a hot solution in spirit of acetate of lead is added as long as a precipitate is produced. Ultimately a little ammonia is added, and the solution is filtered hot from the precipitate. The latter contains the bodies here to be considered.

The crude Lead Compound, to be hereafter referred to as 'Dark Lead Salt,' Ox-Cerebrins.—This body was exhausted with boiling spirit, the extraction being repeated an indefinite number of times; all that which spirit extracted will not be considered any further in this place. The matter insoluble in spirit was now extracted with benzol until nothing further dissolved. There remained insoluble a lead compound which had a peculiar ash-grey colour, reminding the observer at once of the probability that a small part of the lead had by some means or other been converted into sulphide.

Quantation of Lead and Organic Matter and of Sulphur and Phosphorus in the Dark Lead Salt.—(*a*) 1·2992 g. decomposed with hydrothion in hot spirit gave 0·3490 PbS, equal to 23·27 per cent. Pb, and therefore about 76·73 per cent. of organic matter. (*b*) 1·4826 g. burnt with soda and mercuric oxide in a tube, etc., gave 0·1749 $BaSO_4$, equal to 1·62 per cent. S, and 0·1574 pyrophosphate of magnesia, equal to 2·97 per cent. P.

Attempt to isolate the sulphurised Principle, the Presence of which was demonstrated by the foregoing Analysis.—The alcoholic solution which had been obtained in analysis (*a*) of the foregoing paragraph deposited much organic matter on cooling; this was redissolved by heat, and in the hot solution a precipitate was produced by barita water. The precipitate was exhausted by boiling spirit, and the extracts were disregarded. The matter insoluble in spirit was dissolved in benzol, and the solution separated from the insoluble portion. The benzol solution was concentrated by distillation, and precipitated by absolute alcohol. The precipitated body was dried and analysed.

0·3068 (being almost the entire product of this first operation) fused with nitre-flux, etc., gave 0·0288 $BaSO_4$ = 1·32 per cent. S.

This experiment proves that the sulphurised principle contained in the lead salt can be transformed into a barium salt soluble in benzol. This salt contained less sulphur than the original lead salt. When it is compared to other sulphurised barium compounds of similar properties, which I shall have to describe below, it is seen that in the former the sulphur amounts to a much lesser percentage than in the latter. From a third series of observations to be related, it will be seen that the brains of young animals contain at least one sulphur body, which is so labile that on standing in its ether solution it deposits *sulphur* in the metalloid

state, and in crystals too, which are needles as well as octahedra. We have therefore three distinct aperçus, proving the presence of sulphur compounds (other than albuminous ones) in the brain.

Decomposition of the Dark Lead Salt (*Ox-Cerebrins*) *by Oxalic Acid in boiling Spirit.*—It was evident that for the study of the sulphur compound in the lead salt the application of sulphuretted hydrogen, although apparently quite successful in the above experiment, would have to be avoided. A quantity of the salt was therefore boiled with double that amount of crystallised oxalic acid, which theory indicated as necessary for the transformation of all lead into oxalate, in a flask fixed to a refluent cooler, until the salt appeared white and all grey colour had disappeared. The solution was now filtered from the precipitate.

Insoluble Lead Salt (*Oxalate*).—It was exhausted with spirit, then washed with boiling water, suspended in water, and treated with dilute nitric acid. On heat being applied a reaction ensued, the oxalate dissolved, and a small quantity of lead sulphate remained insoluble. It weighed 0·0348 g., equal to 0·0037 g. S in 20 g. salt, or 0·0185 per cent. S in lead salt. This shows that of the 1·62 per cent. S contained in the lead salt, only a minute proportion can be present as sulphuric acid, or in the shape of a metallic sulphate. Possibly even the small quantity found was derived from the oxidation of the black sulphide, which gives to the salt its dark colour.

The Organic Matter from the Lead Salt (total mixture of at least three bodies) was crystallised from the spirit containing the excess of oxalic acid, washed, and pressed. It was next recrystallised from spirit, and appeared white and voluminous. It was analysed in order to ascertain whether it contained sulphur and phosphorus, with the following result:

(*a.*) 0·8668 fused with flux and caustic soda gave 0·0340 $BaSO_4 = 0{\cdot}54$ per cent. S.

(*b.*) The solution filtered from the $BaSO_4$ gave, further, 0·0638 pyrophosphate of Mg, equal to 2·06 per cent. P.

The organic mixture was now extracted with cold benzol, and filtered by air-pressure through a tubular filter. The benzol extracted a small quantity of a body which was recrystallised from spirit and crystallised in needles. The part insoluble in benzol was also recrystallised from spirit. It was then recrystallised from hot benzol, and deposited entirely on cooling. After having

been freed from benzol it was dissolved in about a litre of absolute alcohol, and cooled to 38°. A body was deposited in heavy *sphero-crystals*, and adhered to the glass, so that the solution could be easily decanted clear from it. This was repeated, and all crystals were collected. They represent the body which in the following is termed *spherocerebrin.*

4. Spherocerebrin.

The name is intended to indicate that the body is a cerebrin crystallising in sphero-crystals. It was twice crystallised fractionally from absolute alcohol. The solution having been filtered clear, was now allowed to cool, while the temperature was read off by an immersed thermometer; it began to form a deposit at 50°, of which the main bulk came down between 43° and 41°. When the deposition had ceased, the spherocerebrin was filtered off.

Seen under the microscope, it appears in round balls of very uniform size, which all have a peculiar three-branched mark in the round field. When the balls are rolled, it is seen that they contain three wedge-shaped fans each; the side of such a fan shows radiating needles; three such wedges are united with their sharp straight edges at a line representing the diameter of the ball. By gentle pressure the balls break almost regularly into the three wedge-shaped segments.

Spherocerebrin with oil of vitriol, on standing, gives only a very feeble reddish colour attached to flakes. It is free from sulphur, and contains only unweighable traces of phosphorus (when 0·4462 were analysed). On elementary analysis it gives data which are collated in the following synopsis:

	Percents.	Atoms.
C	62·75	58
H	11·08	123
N	1·23	1
O	24·94	17·3
	100·00	

In its main features spherocerebrin resembles cerebrinic acid, but it contains over this (theory $C_{59}H_{115}NO_9$) an excess of 8H and 8O, or 8 hydroxyls. Spherocerebrin differs, therefore, from cerebrinic acid not only by its crystalline shape, but also by its percentic composition.

5. Principal and Second Product from Dark Lead Salt.

The term 'principal' here refers to quantity; 20 g. of lead salt gave 15·49 of organic product.

The absolute alcohol solution had been filtered at 40°. At 37° cumuli of clouds formed under the bulb of the thermometer. At 36° the entire liquid was turbid. At 35° all translucency was lost, and the crystals projected from margin into fluid. At 34° the surface became concave, and the marginal ring of deposit was out of the fluid. The particles aggregated, and the fluid became again translucent. At 32° clouds of deposit sank, and the temperature remained stationary for some time. At this point the deposit was separated by filtration.

At 30° to 29° a new cloudiness arose, and a deposit began to adhere to the glass. This second deposit included almost all of the matter which was in solution in the alcohol. These processes, therefore, resulted in the separation of the organic matter present in the lead compound in the greatest quantity, into two bodies, one of which, *the least soluble in alcohol*, was recrystallised by itself; while the other, *the most soluble in absolute alcohol*, was also recrystallised by itself.

We have, therefore, at this stage the organic matter in the lead salt separated into the following five products, arranged in the order of decreasing quantities:

(1)	Principal product (not yet unitary) - -	9·59 g.
(2)	Second product (more soluble than foregoing)	4·04 ,,
(3)	Spherocerebrin (least soluble in alcohol) -	1·50 ,,
(4)	Needle body soluble in benzol - - -	0·36 ,,
(5)	Sulphurised compound - - - - -	—

The Principal Product from Dark Lead Salt (*Ox-Cerebrins*).—Water expelled at 90° = 3·4800 per cent. This body is a mixture of a phosphorised principle with at least one cerebrin-like body. If we assume all the phosphorus to be present in the shape of 1 molecle of dineurostearyl-glyceryl-neuryl-phosphatide ($C_{44}H_{83}NPO_8$), and deduct this molecle from the complex of molecles obtained as an empirical formula with P = 1, then we obtain a residue with more than 7 atoms of nitrogen, which, divided by N = 1 (*i.e.*, by 7·36), gives a formula not unlike a cerebrin body with 3 nuclei of neurostearic acid, but with a quantity of oxigen which is too large to satisfy that hypothesis simply.

Synopsis of the Percentages found and the Hypotheses applied.

	Percents.	÷ At. Wgts.	÷ P = 1.	Residue, $C_{44}H_{83}NPO_8$ deducted.	÷ N = 1.
C	59·28	4·94	449	405	55·02
H	10·09	10·09	917	843	113·3
N	1·30	0·092	8·36	7·36	1
P	0·37	0·011	1	0	0
O	28·96	1·81	164	156	21
	100·00				

We have, therefore, in the cerebrin residue of this mixture a still larger number of atoms of oxigen than in spherocerebrin; and this fact may ultimately lead to an explanation of the *acid* character of these compounds, which enables them to form firm compounds with bases, such as the cerebrosides of the phrenosin type do apparently not form.

The second (more soluble in absolute alcohol) product, weighing 4·04 g. (see the list above given), has not yet been analysed. It is not known whether it is or contains the sulphurised principle, the existence of which in the mixture has been proved above. This inquiry must be left to the future.

C. SUBGROUP OF CEREBROSULPHATIDES OR NON-ALBUMINOUS IMMEDIATE PRINCIPLES CONTAINING SULPHUR AS AN ESSENTIAL INGREDIENT.

Introduction. — *A sulphurised principle* had already been discovered in the lead salt from ox-cerebrins, regarding which some preliminary information has been given in a previous chapter. I may also here repeat the notice of an interesting observation already alluded to on p. 182, namely, the deposition of metalloid *crystallised sulphur* from extracts of brains of young animals. I have lately again expressly tested the phosphorised substances which I have isolated and named, and found that they do not contain sulphur as an essential constituent. A gramme of pure kephalin gave only an unweighable trace of barium sulphate; and so with myelin and lecithin. The sulphur-compounds to be described in the following were educed with the aid of barita.

Human mixed Cerebrins.—Barita Process applied for their Separation.—I applied the barita process in this case in the hope that,

as others had by its means obtained cerebrin matter free from phosphorised matter, I might be equally successful. My experiment was, however, so far different from that of others, that I carefully avoided the possibility of the barita acting chemolytically upon the cerebrin-substances. The barita was therefore added only as long as it produced a precipitate, and never in excess, so as to be in solution in any appreciable quantity. I may at once state that in no single instance out of four experiments were the cerebrins obtained free from phosphorus.

The Process.—600 g. of human mixed cerebrin-substances were dissolved, each 100 g. in 3 litres of hot alcohol. To the boiling solution barita-water saturated at the ordinary temperature was added in a thin stream, so as not to interrupt the boiling of the alcohol. Each 100 g. had 450 cc. barita-water added. The mixture was boiled for a few moments, and then the solution decanted from the adhesive *precipitate.* The latter became hard on cooling, and was detached and powdered. It was now exhausted with boiling alcohol; many extractions and pulverisations were required to withdraw all the matter which alcohol would redissolve.

Nature of the Precipitate produced by Barita.—The barita-water does not produce a precipitate in the hot alcoholic solution of the cerebrin mixture by virtue of its water, as was specially proved by a blank experiment with water only. It precipitates, in the first place, *in combination with barita,* a body (or mixture of bodies) which is soluble in cold benzol, and which will be treated of immediately. At the same time there fall down portions of the several cerebrin bodies, phrenosin, kerasin, etc., of which another portion remains in solution, and which are then again extracted from the precipitate by boiling alcohol without retaining more than traces of barita, precipitable by carbonic acid from the alcoholic solution. These latter bodies are insoluble in cold benzol, and separable thereby from the stearoconote or mixture of barita compounds.

It must at once be pointed out that the matters soluble in cold benzol cannot be separated from those insoluble in benzol by one operation only. When benzol has acted upon the powdered particles of the precipitate, they seem to be covered by a layer of matter insoluble in benzol, which prevents this solvent from reaching the matters soluble in it, which are contained in the

interior of the particles. It is therefore necessary to extract the powder alternately with cold benzol and hot alcohol, and sufficiently often until all the matter is dissolved in hot alcohol and cold benzol respectively, or remains insoluble in either.

Matters soluble in cold Benzol.—The benzol solution obtained in the manner described in the foregoing is clarified by repose, filtration, and decantation, until on standing further it remains perfectly clear and brilliant. It is then concentrated to a small bulk, with the precaution of keeping the solution perfect. It is now treated with absolute alcohol as long as a precipitate or turbidity is thereby produced. The precipitate is further extracted by boiling absolute alcohol, and dried in vacuo over oil of vitriol. The alcoholic mother-liquors, particularly those obtained by boiling, contain a quantity of a body which appears in curved needles, and therefore will be designated meanwhile as *curved needle body.*

The Barita-Compound soluble in cold Benzol and insoluble in Alcohol.—It was dried over oil of vitriol, at 70° in air-bath before analysis. It was a coloured powder, and on elementary analysis gave data which are collated in the following synopsis :

Synopsis of Data :

Elements.	Percents.	÷by At. Wgts.	÷by S = 1(0·125).
C	30·86	2·5716	20·57
H	4·88	4·88	39·
N	0·74	0·0528	0·42
S	4·00	0·125	1·
Ba	35·30	0·257	2·
P	2·55	0·082	0·65
O	21·67	1·354	10·
	100·00		

The study of the relations of the atoms to each other gives at once some interesting information. The sulphur is to barium as 1 : 2, a fact which became already apparent in the course of the analysis. Carbon and hydrogen are to each other as 1 : 2 very nearly, indicating the presence in the compound of radicles of the fatty series. Phosphorus and nitrogen stand apparently in no relation to each other, and in none to the sulphur or barium. The body is unquestionably a mixture, but may be of substances having some analogy with each other. At least the bearing of the product towards solvents would support such an hypothesis.

Several smaller preparations obtained in a manner similar to the

process described in the foregoing were analysed, and gave, one, 32·97 per cent. Ba, 1·06 per cent. S, and 1·15 per cent. P; the other, 22·14 per cent. Ba, and 1·95 per cent. S. They evidently contained a lesser proportion of the sulphurised body than the main preparation.

D. SUBGROUP OF AMIDOLIPOTIDES, OR NITROGENISED FATS,

1. Bregenin: its Isolation and Properties.

I have isolated the principle from both human and bovine brains. It crystallises, fuses like a fat, and contains nitrogen. It is extremely soluble in several reagents, and does not combine with acids, alkalies, or salts.

Mode of Isolation.—Human cerebrins in hot alcoholic solution were treated by barita-water. The precipitated cerebrin bodies were freed from barita by sulphuric acid in alcohol and recrystallised. The most soluble part contained the bregenin. From this, sphingomyelin was removed by cadmium chloride; the resulting solution was now evaporated to dryness and extracted with cold benzol; kerasin remained undissolved, while bregenin and some other matters dissolved in the benzol. The matters dissolved in benzol were treated with boiling ether in a suitable apparatus, when a body remained insoluble in boiling ether, while two bodies dissolved; one was deposited from the ether on cooling—*krinosin*, to be described below—while another remained dissolved in the cold ether, namely, *bregenin.* After removal of the ether the bregenin was dissolved in a minimum of watery spirit, filtered hot, and allowed to crystallise. When after repeated crystallisation, removal of the mother-liquor by pressure between bibulous-paper, etc., the product consisted of white microscopic leaflets and curved needles only, it was considered pure and analysed. Its solution in spirit does not give precipitates with cadmium or platinum chloride, or with lead acetate, with or without ammonia. As long, therefore, as in a supposed or reputed solution of bregenin such precipitates are produced, they have to be removed as impurities. It is much more soluble in absolute alcohol than in watery spirit; from the former it is deposited as a white solid mass, when the solution is concentrated; when dilute no deposit at all may take place; it is useful to dilute the hot alcoholic solution with boiling water until a permanent

turbidity is produced, and allow the mixture to cool. Good crystals are obtained from such a solution.

From a concentrated spirit solution bregenin crystallises between 50° and 25°. At 30° it is not completely deposited. A dilute spirit solution becomes hazy about 40°, and begins to crystallise only at 25°. When thus deposited slowly it appears in balls and irregular masses, without any curved needles. These balls can be transformed into needles by resolution in spirit and rapid cooling down of the solution.

Physical and Chemical Properties of Bregenin.—Besides the characters already described in the foregoing, bregenin has the following diagnostic properties. When heated in a water-oven it becomes a little coloured, and then fuses below 98° to an oily fluid. On cooling it solidifies to a hard mass, which is not plastic like fat or wax, but splinters, when cut, in all directions, and is highly electrical. Its fusing-point is between 62° and 65°. When the fused and recongealed body in the filiform tube is again heated, it becomes in part transparent about 62°, but a core remains opaque until a higher temperature is reached, when all is again fused. This is due to the viscosity of the fused body. At the lowest fusing-point it is transparent, but so viscous as to be hardly mobile, or only very slowly mobile; with rising temperature it becomes as fluid as a molten fat, and the interval between the point of fusion and the point of greatest liquidity is considerable. While it fuses and coalesces in the narrow tube at 62° to 65°, it flows down the sides of the tube only at 75° to 76°, and then, on cooling, sets with a sudden appearance of opacity at 58°. These experiments were made with the purest specimen, which had been kept in a fused state in the water-oven for hours. The bearing of bregenin with water is very remarkable. When heated with much water, it fuses like a fat, on the top of it. But on agitation it increases in bulk and becomes viscogelatinous. This hydration, which is completed only on long standing in the water, causes it to increase in bulk considerably. When the swelled mass, after decantation of all water, is heated on the water-bath, it contracts, gives out water, and fuses as in its original state while all water is being evaporated.

With oil of vitriol it gives no purple on standing; the solution remains a little yellow. When sugar-syrup is added to this solution it becomes perfectly white, and gives no vestige of

purple. From this it is highly probable that bregenin does not contain the radicles either of oleic acid or of sphingosin.

The fused bregenin, when quite cold again, can be powdered. In this state it was subjected to elementary analysis, which yielded the following data :

Synopsis of Analysis and Theory.

	1.	2.	3.	4.	5.	Mean.	÷At. Wt.	÷N=1.
C	73·59	73·69	73·72	—	—	73·66	6·138	C_{40}
H	12·54	12·92	12·56	—	—	12·64	12·64	H_{81}
N	—	—	—	2·22	2·15	2·18	0·155	N
O	—	—	—	—	—	12·52	0·781	O_5
						100·00		

This body, *bregenin* (from the Low German ' bregen,' head or brain, the latter English word being probably a contraction merely of bregen), $C_{40}H_{81}NO_5$, is thus shown to be approximate in the number of its carbon atoms to both the mononitrogenised phosphatides and to the cerebrosides. From the latter it is sharply distinguished by the low amount of oxigen which it contains. It has almost exactly the composition of the lowest phosphatide of the lecithin group minus the phosphoric acid, for

$$C_{40}H_{81}NO_5 + PH_3O_4 = C_{40}H_{83}NPO_8 + H_2O.$$

This might make us suspect that it was derived from such a group by the mere loss of phosphoric acid, and consequently that it might be a product and not an educt. But such a mode of decomposition of a phosphatide has no analogy in the decompositions which have thus far been artificially produced. For in these phosphorised bodies when they contained glycerol, this alcohol remained mainly with the phosphoric acid. Supposing, as a mere hypothesis, that bregenin did contain glycerol, then there would be no room for neurin as the nitrogenised radicle. In the absence of neurin, the nitrogenised radicle would have to be one which does not occur in the mononitrogenised phosphatides, as far as they are known, and this would negative the suggestion that bregenin might be derived from such a phosphatide by loss of phosphoric acid. Besides, such a hypothesis also presupposes that the binding radicle is an alcohol like glycerol, and that phosphoric acid is outside the nucleolar arrangement, and merely attached as a side-chain—a theory which is clearly impos-

sible for those phosphatides, which, as I have shown, contain no glycerol, and is therefore not probable for the others which contain it.

Bregenin is obtained by the foregoing processes in small quantities only, and a great number of preparations were required to yield material sufficiently pure for analysis. It is evident that the greater part of the bregenin present in the brain must, in the process of extraction, pass into the alcoholic and ethereal solution containing lecithin and kephalin; then it must at last remain with the cholesterin, where we accordingly find it. And it is separated from this only by treatment with caustic potash, which chemolyses and removes the products of bregenin, while leaving cholesterin unaltered. It will therefore be seen that much further study will be required to elucidate the physiological quantities and functions of this remarkable substance.

2. Krinosin, the Second Representative of the New Series of Nitrogenised Fats, or Amido-Lipotides: its Isolation and Properties.

Mode of preparing Krinosin.—Dry, finely-powdered crude kerasin as obtained from the process for the isolation of sphingomyelin by cadmium chloride is exhausted in the ether extraction apparatus by boiling ether. The solvent should be perfectly anhydrous, and be renewed from time to time until it extracts nothing more. For the extraction is but slowly completed, and in most cases requires the apparatus to be kept in action for several days. The hot ether solution deposits krinosin on cooling and standing as a voluminous felted mass of long microscopic fibres, a wilderness of mere lines, mostly without visible end. If the vessel has not been agitated during the cooling process, the felted mass forms a complete jelly with the ether. By strong agitation this structure is destroyed, and there remain only a few lumps of felted matter, looking like bluish paper pulp in an abundance of water. The fibres, having been collected on a filter, dry to a hard mass, when moisture, so easily collected by the cold produced in the course of the evaporation of the ether, is allowed to deposit upon them; when only little moisture has been in contact with them, they dry to a white spongy matter, which is just a little waxy on compression or when rubbed with the finger-nail. When the fibres, taken out of the anhydrous ether, are dried in vacuo over oil of

vitriol, they become a perfectly white and pulverisable mass. When this powder is now heated in a water-oven to 98° for some time, it becomes somewhat plastic and assumes a yellowish colour. After cooling it is again hard and perfectly pulverisable, but retains the colour acquired by the heating. Krinosin is insoluble in cold, easily soluble in boiling alcohol. It gives no purple reaction with sulphuric acid alone, and none with sulphuric acid and cane-sugar. It is consequently not a cerebroside, and does not contain any oleo-cholide radicle.

Synopsis of the Means of Elementary Analyses and Theory of Formula.

	Percentages.	÷At. Wgts.	÷N = 1.
C	70·94	5·911	38·1
H	12·28	12·28	79·
N	2·17	0·155	1·
O	14·61	0·913	5·8

Consequently the nearest formula warranted by the quantations is $C_{38}H_{79}NO_5$. Leaving out of consideration a slight excess of hydrogen, probably due to the substance not having been dried at a higher temperature, but only in vacuo, there seems good reason to suppose that krinosin is a homologue, in an isomeric series, of bregenin, for, we have

Bregenin - $C_{40}H_{81}NO_5$
Krinosin - $C_{38}H_{77}NO_5$.

The homology is probably not in the same series, as the higher carbonised bregenin fuses below 70°, while krinosin does not fuse below the heat of boiling water.

E. SUBGROUP OF ALKALOIDS.

1. Alkaloids from the Human Brain.

1. *Hypoxanthin.*—The *deposit* from the concentrated solution of the extractive matters of the brain soluble in water was exhausted with HCl; the solution was treated with excess of silver nitrate, and the precipitate extracted to exhaustion with boiling dilute nitric acid. The deposited hypoxanthin silver nitrate was isolated and added to the preparation obtained by the following process.

The *extract* was diluted and precipitated with mercuric acetate. The precipitate was washed by repeated levigation in water, and decomposed by H_2S. The HgS was repeatedly extracted with boiling water. The solutions were evaporated to a thick dark syrup, and filtered. A first crop of impure hypoxanthin remained on the filter. Addition of ammonia produced a second one.

Both portions were united, dissolved in dilute HCl, boiled with animal charcoal, and reprecipitated by ammonia ; evaporation to dryness and extraction of the ammonium chloride with water left the hypoxanthin, which was now also transformed into silver nitrate salt.

The united silver nitrate salts were treated with ammonia during several days, and washed with ammonia-water on the filter. The solutions contained little besides ammonic nitrate. The last extracts contained a very small quantity of a body soluble in ammonia, precipitated in flakes on evaporation, and re dissolved in nitric acid on boiling, less readily precipitated on cooling than hypoxanthin salts, and therefore more like xanthin or guanin.

The silver hypoxanthin was decomposed by H_2S, but the solution remained black and unfilterable. It had to be boiled with animal charcoal, and then became almost colourless. But the charcoal retained much hypoxanthin, and had to be boiled with many new portions of water before the whole of the base which could be extracted was obtained. Some was no doubt lost in the charcoal.

The solution was acid and contained some phosphate from the charcoal. It was treated with a little ammonia, filtered, evaporated to dryness, and extracted with cold water. The *pure white crystallised hypoxanthin* was collected on a filter, washed with cold water, and dried. It formed crystalline white masses and granules.

2. *Second Alkaloid.*—The mother-liquor of the hypoxanthin, treated with barita to expel the ammonia, and then with carbonic acid, retained a large amount of barita in solution. The solution gave precipitates with ferric chloride on boiling, and with phosphomolybdic acid. The barita was removed by sulphuric acid, and the acid solution precipitated by phosphomolybdic. The precipitate was decomposed with barita; the filtrate was refiltered three times during concentration.

The solution was acidified with HCl, and precipitated by $AuCl_3$. The *auric chloride salt* was washed with water (the salt was very

soluble in excess of HCl), and put in a vacuum to dry. On analysis it gave the data contained in the following synopsis :

Synopsis and Computation of Analyses.

	Percentages.	÷ by At. Wgts.	÷ Au = 1.	N = 1.
C	19·447	1·620	6·98	2·38
H	2·221	2·221	9·57	3·27
N	9·499	0·678	2·92	1·00
O	7·890	0·493	2·12	0·72
Au	45·743	0·232	1·00	0·34
Cl	15·200	0·428	1·84	0·63

It is at once evident that the compound contains more gold than could be present in the form of terchloride; for the 15·200 per cent. Cl require 28·073 Au; the excess of Au, therefore, amounts to 17·670. This may be supposed to have been present in the reduced metallic state. The salt is, therefore, one of those unstable compounds which decompose during isolation and drying. Nevertheless, the consideration of the composition of the residue will afford some, the only, means of judging of the composition of the original alkaloid. From the computation of the figures obtained by N = 1, and deducting the excess of gold, and referring the possible organic molecle to a molecle of $AuCl_3$ (which requires the multiplication of the quotients by N = 1 by 5), we get $C_{11}H_{16}N_5O_{3\cdot5}AuCl_3$. But calculating the salt which remains after deducting the excess of gold as a hydrochlorate and aurochloride, containing 4Cl to 1Au, we get—

	Percentages.	÷ At Wgts.	÷ Au = 1.
Cl }	15·200	0·428	4·00
Au }	21·055	0·107	1·00
C	19·447	1·620	15·14
H	2·221	2·221	20·75
N	9·499	0·678	6·33
Au	24·688	—	—
O	7·890	0·493	4·60

leading to a formula consonant to the general chemical theory of gold double salts of $C_{15}H_{20}N_6O_5,HCl,AuCl_3$.

3. *Third Alkaloid.*—This was left in the mother-liquor after removal of the two previous bodies, and precipitated by phosphomolybdic acid from the acidified liquid. The treatment of the precipitate by barita yielded the alkaloid in solution, retaining barita not precipitable by carbonic acid. The solution was precipitated by absolute alcohol. It remained a syrupy coloured

liquid, and did not crystallise after months of standing. It had a marked smell of human sperma. It was so small in quantity that no further research could be instituted upon it. After it had been removed from the mother-liquor representing the extracts obtained by the mercuric acetate precipitation, there seemed to be no further substance of an alkaloidal nature contained in them.

2. Alkaloids contained in Ox Brain.

The presence of the alkaloids in the water-extracts of brain-matter is indicated by the precipitates which they give with $AuCl_3$, I in KI, $HgCl_2$ in KI, picric, tannic, and phosphomolybdic acids.

They may be obtained as shown in the preceding pages by the application of the phosphomolybdic acid process, $HgCl_2$ process, and collaterally by the basic lead acetate process (for inosite). The latter process, however, does not yield them if preceded by the phosphomolybdic acid process. They may be separated when in the free state by evaporation to a syrupy consistency when the hypoxanthin is deposited while the second base is uncrystallisable. The same remarks apply to a mixture of the hydrochlorates, the hypoxanthin compound in this case being obtained in a crystalline form. Neither the watery solution of the free hypoxanthin nor that of its hydrochlorate gives a $PtCl_4$ precipitate. The hypoxanthin may be purified by dissolving in boiling water and addition of excess of silver nitrate and nitric acid. The precipitate so formed gradually dissolves in hot nitric acid, and from the filtered solution deposits on cooling in a mass of homogeneous needles. Obtained in this way it is pure, as testified by the following analysis:

Analyses and Theory.

	Percentages.	÷ At. Wgts.	÷ Ag = 1.
C	= 19·746	1·645	5·2
H	= 1·539	1·539	4·8
N	= 23·516	1·679	5·3
O	= 21·099	1·318	4·1
Ag	= 34·100	0·315	1·0

$$= C_5H_4N_4O + AgNO_3.$$

A combination of the hypoxanthin with silver was also obtained by precipitation of its solution with ammoniacal silver nitrate;

it contained 60·46 per cent. silver, whereas had it been pure $C_5H_4N_4OAg_2$, it should have contained 61·7 per cent.

The hydrochlorate of the second base is soluble in alcohol but precipitated by ether; on the whole it is a perishable compound. In one experiment where the hypoxanthin had been removed from a mixture of the two bodies, this second base was again passed through the phosphomolybdic process; the product gave a precipitate with $AgNO_3$ in the presence of HNO_3, which proved to be a further quantity of hypoxanthin salt. Thus purified the second base was again passed through the phosphomolybdic acid process, then converted into gold chloride salt and analysed; it yielded—

C	27·37
H	3·71
N	11·88
O	13·836
Au	31·864
Cl	11·34
	100·000

Computation of Analyses.—In this compound the gold stands to the chlorine as 1 : 2; deducting now the gold and chlorine and recalculating the percentages, we have:

Percentages.	÷At. Wgts.	÷N=1.
C = 48·18	4·015	2·7
H = 6·53	6·530	4·3
N = 20·92	1·494	1·0
O = 24·37	1·523	1·0

$$= C_3H_4NO.$$

F. SUBGROUP OF AMIDO-ACIDS AND IMIDES.

1. Leucin and Allied Bodies; Tyrosin.

The filtrates from the mercuric precipitates were freed from mercury by hydrothion, and evaporated to a syrupy consistence; the syrup was treated with absolute alcohol, and thereby separated into a portion soluble, and another insoluble, in alcohol.

Portion Soluble in Alcohol.—The alcohol was evaporated, the residue treated with neutral plumbic acetate. The precipitate consisted mainly of phosphate and chloride, and was removed. After this, basic plumbic acetate was added to the filtrate, and

the precipitate collected and decomposed with hydrothion; the concentrated filtrate from the plumbic sulphide on treatment with absolute alcohol gave a crystallisation of *inosite.*

The mother-liquor of this inosite, freed from alcohol by evaporation and acidified, gave a small precipitate with phosphomolybdic acid, thus proving that the mercuric acetate had not removed all the alkaloid, but left some, which was precipitated by basic lead acetate (*third alkaloid*).

The lead was now removed from the mother-liquor by an excess of sulphuric acid, and the acid liquid extracted with large quantities of ether at intervals. The ethereal extracts, after removal of the ether by distillation, left a mixture of acids; of these, acetic acid, introduced with the mercury and lead salts, was evaporated on the water-bath. The remaining syrup consisted mainly of *lactic acid*, but also contained some *succinic acid*, as will be described more fully lower down.

Portion Insoluble in Alcohol.—This, on solution in a minimum of water, retained a quantity of *solid matter* in suspension, which was separated by filtration. The residue on the filter was pressed, and on treatment with cold water gave to this solvent a matter recognised as *leucin and allied bodies.* The matter insoluble in cold, but easily soluble in hot water, was *tyrosin.*

The leucin was purified by precipitating the coloured impurity out of its watery solution by means of mercuric nitrate, removing the excess of mercury by hydrothion, evaporating the solution, precipitating the acid liquid by ammonia, collecting and pressing the precipitate in bibulous paper, and recrystallising it from spirit. The first crystals were pure leucin; the second crystals were, however, a different body, more soluble than leucin, and not easily separated from leucin, on account of the similarity of properties. The last mother-liquor dried up, leaving a trace of matter.

The tyrosin was purified by hydrochloric acid and charcoal, and precipitation with ammonia.

The mercuric nitrate precipitate from leucin was also decomposed, and seemed to contain only a small amount of alkaloid No. 2.

Note on a Peculiar Potassium Salt.

The liquid part of the portion insoluble in alcohol was diluted with water, and treated with neutral lead acetate; the precipitate,

decomposed by hydrothion, gave a syrup which, treated with absolute alcohol, deposited a peculiar *viscous colourless potassium salt;* this was isolated, and, on account of its peculiar nature, the presence of the potassium was specially proved by combustion and platinic chloride. The part of the syrup soluble in alcohol was not further examined. The filtrate from the neutral lead acetate precipitate was treated with basic lead acetate, and the new precipitate decomposed with hydrothion; the concentrated product was treated with absolute alcohol, and gave white *inosite,* partly anhydrous, partly hydrated. In the mother-liquor of this inosite nothing, apparently, but some alkaloid No. 2 remained. The mother-liquors from which the foregoing bodies had been extracted and precipitated were freed from the impurities introduced as reagents as far as possible and evaporated, and then formed a nearly colourless, viscid, uncrystallisable mass; this was distilled in superheated steam, but yielded no glycerol. It gave a precipitate with phosphomolybdic acid, from which a syrupy alkaloid, smelling like sperma, was obtained (alkaloid No. 3).

V.

GROUP OF IMMEDIATE PRINCIPLES, COMPOSED OF THREE ELEMENTS ONLY: ALCOHOLS, CARBOHYDRATES, AND ACIDS.

A. SUBGROUP OF ALCOHOLS.

CHOLESTERIN.

CHOLESTERIN is present in the brain in very large quantity. In the process for the separation of the brain principles above described it passes mainly into the solution containing the kephalins; these bodies have to be precipitated by lead; myelin the same; the other phosphatides have then to be removed by cadmium chloride. At last a mixture of cholesterin, with several other bodies, amongst them bregenin, is obtained; this mixture must now be boiled with caustic potash in alcohol, to decompose the admixtures, and retain their decomposition products in solution while cholesterin crystallises. It is pressed and recrystallised as often as necessary to give it its brilliant appearance, and its melting-point, 145°. It is then the same body as that obtained from human gallstones. It crystallises from spirit as monohydrate, $C_{26}H_{44}O + H_2O$, and loses the water at 100° or in vacuo. While from spirit it crystallises in rhombic plates, it is deposited from chloroform or benzol in anhydrous needles. It rotates polarised light to the left. Ether solution at 15°: $[\alpha]D = -31{\cdot}12°$; in chloroform solution: $[\alpha]D = -36{\cdot}61°$. It is insoluble in water, little soluble in cold watery spirit, easily soluble in from 5 to 9 parts of boiling alcohol, the more the stronger the alcohol is. It can be distilled in a vacuum unchanged at a temperature of 360°. On distillation by heat under ordinary air-pressure it is partly transformed into hydrocarbons. It combines with organic acids

when heated with them under pressure. When oxydised by permanganate in acetic acid solution it yields cholestenic acid, $C_{25}H_{40}O_4$, and similar acids with 5 or 6 atoms of oxigen. With nitric acid it yields cholesteric acid, $C_{12}H_{16}O_7$, which is remarkable, as it is also formed by nitric acid from cholic acid, and thus it establishes a relationship between cholesterin and biliary acids. With bromine cholesterin gives a product of addition, $C_{26}H_{44}O,Br_2$; with concentrated sulphuric or phosphoric acid it yields a number of hydrocarbons, cholesterylens, which are isomeric with each other.

Cholesterin gives some very characteristic reactions. Treated with concentrated sulphuric acid and a little iodine, it becomes violet, blue, green, and red in succession. This reaction is useful for recognising cholesterin under the microscope. When a little cholesterin is evaporated with a drop of nitric acid at a gentle heat, a yellow spot remains, which, if covered while warm with a drop of ammonia, becomes red; this red is not altered by the addition of fixed alkali.

Reaction of Cholesterin with Oil of Vitriol and Chloroform; Spectral Phenomena of the Product.

The cholesterin was obtained from the brain, and fused at 147° C. A portion was dissolved in chloroform, and an equal bulk of oil of vitriol added; this produced a dark red coloration both in the chloroform and in the acid.

The chloroform solution thus obtained presented the following spectra:

The most concentrated solution obscured all but the red.

After it had been a little more diluted one broad band appeared in yellow and green, and another in green to blue, but it had only about half the intensity of the other.

When it was still more diluted the broad band split into two bands, the band in green to blue remaining.

The solution when poured into a dish became rapidly blue, green, and at last colourless.

On evaporation to dryness and re-addition of sulphuric acid to the residue, a slight restoration of a dirty red colour took place, but with chloroform no restoration; the permanent colour of the residue was a light green.

Reaction of Cholesterin with Oil of Vitriol and Glacial Acetic Acid.

The dark red-brown oil of vitriol solution of cholesterin when thrown into glacial acetic acid dissolves entirely to a dark red solution almost impenetrable to light. This solution presents the following spectra:

Through the concentrated liquid only red passes; when it is more diluted two bands appear, one feeble, in red, the other strong, in orange and yellow.

In the still more diluted liquid three bands appear, the solution being brilliant; the third band is broad, and reaches to F.

Hence the middle band of the chloroform solution was absent from this; the broad band of the chloroform solution was there, but much stronger and broader. There was a powerful green fluorescence. As the third band of the acetic acid solution is new and does not occur in the chloroform solution, at least two coloured bodies are produced in this reaction.

By oxidation with chromic acid in acetic acid, cholesterin yields an acid of the formula $C_{24}H_{39}O_5$, and other products.

Phytosterin, the cholesterin of plants, is the second isomer, $C_{26}H_{44}O + H_2O$; its fusing-point is 132° to 133°; its chloroform solution turns to the left $= [\alpha]D = -34{\cdot}2°$.

Isocholesterin, $C_{26}H_{44}O$ is the third isomer, and occurs in the wool-fat from sheep, together with the ordinary cholesterin. When crystallising from alcohol it forms gelatinous masses; from ether it crystallises in needles. It does not give the reaction with oil of vitriol and chloroform which cholesterin gives. It fuses at 137° to 138°.

Paracholesterin, $C_{26}H_{44}O + H_2O$, is the fourth isomer, and is obtained from the fungus æthalium flavum. Fuses at 134° to 134·5°. It differs from phytosterin only by its rotation being less, namely $[\alpha]D = -28{\cdot}88°$.

The quantation of cholesterin in the brain is connected with many difficulties. It is not impossible that the bearing of cholesterin with benzoic acid under pressure at high temperature, 200°, may be utilised for this purpose. The two bodies combine and form an ether, which is almost insoluble in boiling spirit; but crystallises from ether in peculiar rectangular plates. The benzoate of isocholesterin crystallises in needles. When such plates and needles are obtained mixed, they can be separated by levigation

with spirit. This process therefore offers a means of separating cholesterin from isocholesterin, when they occur mixed with each other, as they do in wool-fat. It may possibly serve in some cases of brain analysis, and for this purpose the reaction should be borne in mind.

B. SUBGROUP OF CARBOHYDRATES.

Inosite.

Inosite occurs in the parenchyma of most tissues of the animal body, but in largest quantity in muscle and the brain. It also occurs in plants, *e.g.* in beans, and in Sauterne wine. It crystallises as a dihydrate, $C_6H_{12}O_6 + 2H_2O$, and does not rotate the ray of polarised light. It does not undergo the alcoholic, but easily the lactic fermentation, and the lactic acid which results is optically inactive. With nitric acid it yields a trinitrited, and a hexanitrited substitution compound, $C_6H_9(NO_2)_3O_6$, and $C_6H_6(NO_2)_6O_6$. Inosite is completely precipitated from its solution by basic lead acetate; the lead compound is decomposed by hydrothion, and the solution evaporated to a small bulk. When to this alcohol is added, crystals of inosite are formed on standing. The following is a good test for inosite. Evaporate the liquid to be tested for inosite in a porcelain dish to the bulk of a few drops, and then add a small drop of mercuric nitrate. This produces a yellowish precipitate. When this is spread as far as possible over the surface of the porcelain, and the dish is further heated with great caution, there remains, as soon as all fluid is evaporated, and provided that no excess of reagent has been added, a residue which is whitish-yellow at first, but soon becomes more or less dark red, according to the quantity of inosite present. The colour disappears when the dish gets cold, but reappears on reheating it gently. If, when the colour has appeared, the dish is overheated in the slightest degree, the mixture undergoes a sudden decomposition, though without incandescence, and becomes black. Inosite is not capable of reducing Fehling's solution. Its bearing with copper salts was little understood before the following observations on the subject were made.

Compound of Cerebral Inosite with Cupric Oxide.—When to a hot solution of inosite (from ox-brain) a saturated solution of copper acetate is added, a light green precipitate immediately ensues.

When copper acetate is added in excess, so that the filtrate has a blue colour, and the mixture is warmed, almost all inosite is precipitated out of the solution. The green precipitate of inosite copper can be heated with pure water, without more than traces of copper dissolving in the water. The solution is colourless, but gives a brown coloration with potassium ferrocyanide and acetic acid. The light green precipitate of inosite copper (*first precipitate*) on being dried in the air-bath at 110°, became dark green, nearly black, and was then analysed. It contained 47·11 per cent. of Cu.

A compound of one molecle of inosite with three molecles of cupric oxide, $C_6H_{12}O_6 + 3CuO$, of which the atomic weight would be $182 + 238 \cdot 2 = 420 \cdot 2$, requires 45·2 per cent..

The compound when *boiled* with water is decomposed at the place where the vessel, whether platinum or glass, is hottest. It is not decomposed when warmed gently on the water-bath. When a solution of inosite is *evaporated* with excess of copper acetate on the water-bath, all inosite becomes insoluble in the shape of the compound described in the foregoing. The excess of acetate may be washed out with warm water, but the precipitate may not be boiled in platinum or glass over the free flame, as it forms a reddish-brown adherent decomposition product. The compound is soluble in acetic acid, with slight coloration, without residue. It is soluble in ammonia, with a deep blue colour. A trace of matter remains insoluble (which is not the case with the acetic acid) and may explain the slight excess of copper found in the analysis.

When this compound was heated on platinum, it showed the following remarkable bearing: it scintillated and deflagrated, while evolving acid fumes. A red residue was left, which on being thrown into the air took fire and burnt (pyrophorus). Probably the three molecles of cupric oxide gave up half their oxigen, and remained as cuprous oxide mixed with some carbonaceous matter.

Second Precipitate.—On addition of more copper acetate to the solution from which the first precipitate had been filtered, and application of a gentle heat, a second precipitate ensued, which was and remained green. Dried at 110° it gave on analysis, mean of three quantations, 44·59 per cent. Cu.

When this compound in the state of powder was heated at the margin, it took fire, and then burned spontaneously through,

leaving a red residue. This residue also was pyrophorous when thrown into the air.

Third Precipitate.—The mother-liquor of the second precipitate was concentrated by evaporation, and formed a third precipitate. This, after isolation, was dissolved in dilute ammonia, and reprecipitated by gentle evaporation. It was light green, and when gently dried was analysed, and found to contain 40·10 per cent. Cu.

A trihydrate of the cupric inosite requires 40·10 per cent. of Cu. That this compound, $C_6H_{12}O_6 + 3CuO + 3H_2O$ (atom. weight $= 474·2$) had really been obtained, was further proved by the loss which it suffered on being dried at 110°, being equal to three molecles of water. For the dry compound gave on analysis 45·38 per cent. Cu, while theory requires 45·2 per cent. Cu.

Fourth Precipitate.—This was obtained after the third, by the same process as the latter. It was bright green, and dried at 100° gave on analysis 45·68 per cent. Cu.

The *inosite from ox-brain* used in the foregoing research was a finely crystallised, on the whole very pure specimen. Nevertheless, the first precipitate was somewhat impure, as indicated by its physical properties and its composition. But the precipitates Nos. 3, 4, were so pure that their analyses yielded almost theoretical results.

It was therefore very surprising that an equally well crystallised and apparently perfectly pure specimen of *inosite from human brain*, on being mixed and treated with copper acetate like the inosite from ox-brain, should yield totally different and greatly varying results.

The *first precipitate*, dried in vacuo over sulphuric acid, lost at 110° 1·53 per cent. H_2O, and contained 47·48 per cent. Cu.

The *second precipitate*, dried in vacuo, etc., lost at 110° 1·77 per cent. H_2O, and contained 51·23 per cent. Cu.

The mixture, on further evaportion, formed a third, and after its removal only an insignificant fourth precipitate, although after addition of copper solution it formed a fifth and a sixth precipitate, which contained more copper than the first precipitate.

The *third precipitate*, dried in vacuo over oil of vitriol, lost at 110° 2·85 per cent. H_2O, and contained 47·73 per cent. Cu.

The *fifth and sixth precipitates*, dried in vacuo over sulphuric acid, lost at 110° 1·34 per cent. H_2O, and contained 49·81 per cent. Cu.

These precipitates, therefore, contained about two, or four, or six per cent. more copper than corresponds to tricupric inosite. When they were dissolved in ammonia and the solution evaporated cautiously, discoloured products were obtained. From these data I conclude that the inosite from the human brain is either altogether different from that contained in the brain of the ox, or is accompanied by another similar carbohydrate of less stable quality. In any case, the subject calls for further investigation.

Inosite is a hexadynamic alcohol, and forms, as we have seen above, two nitrite ethers, a hexanitrite and a trinitrite. Similarly, though probably not by substitution of hydrogen, it forms a tricupric compound; and perhaps at the same time a small quantity of a hexacupric one, if the compounds with more than 45·2 per cent. Cu have not to be considered as combinations with bodies other than inosite.

C. SUBGROUP OF (NONNITROGENISED) ORGANIC ACIDS.

1. Lactic Acid.

The mixed acids were heated on the water-bath until acetic acid was expelled, redissolved in water, and neutralised while hot with freshly prepared zinc carbonate. The zinc salt was crystallised and recrystallised an indefinite number of times, until perfectly white, crystallised throughout, and homogeneous. During these operations a coloured matter became insoluble, and had to be removed by repeated filtration. The crystallised salt was found to be pure zinc lactate, containing the variety of lactic acid known as *lactic acid from flesh, or sarkolactic acid*. Not only did the salts yield the particular amount of water of crystallisation which distinguishes them from the zinc salts of the fermentation lactic acid, but the free acid itself showed the power of polarising light, which is not possessed by the product of the fermentation of milk.

Summary of Analyses of Zinc Lactate from Human Brain.

The salt was dried in vacuo until it lost no longer in weight, and then at 110° until constant. Two specimens showed a loss of water of crystallisation amounting to 12·82 per cent., and 12·90 per cent. This corresponds to the theory of 12·82 per cent. H_2O water of crystallisation.

In the same hydrated salt, dried in vacuo, the zinc was estimated by precipitation with carbonate in the usual manner, and found in two experiments to amount to 23·24 per cent., and 23·28 per cent.; while theory requires 23·35 per cent. Zn.

Summary of Analyses of Zinc Lactate from Ox Brain.

The salt, after having been dried in vacuo over oil of vitriol, lost 12·80 per cent. H_2O at 110°.

The zinc was estimated in the anhydrous salt by precipitation and ignition, and found in two experiments to amount to 26·69 per cent., and 26·69 per cent. Theory requires 26·79 per cent. Zn in the anhydrous salt, $C_6H_{10}O_6Zn$. The formula of the hydrated salt is $Zn(C_3H_5O_3)_2(H_2O)_2$.

Physical Peculiarities of the Lactic Acid from Brain and its Zinc Salt.

When the lactic acid as obtained from the ether extract, a state in which it was yet yellowish and gave out an odour, was decolorised by animal charcoal, and a somewhat concentrated solution of it was placed in a tube, of 220 mm. in length, and containing about 26 cc. of fluid, and subjected to the influence of the polarised ray of yellow light in a Wild's polaristrobometer, it was found to turn the plane of polarisation to *the left* (to the measured extent, in the particular instance of an acid of uncertain strength, of 1° 20′).

The acid was next transformed into zinc salt by boiling with zinc oxide, and the solution of salt was evaporated to the same volume as that occupied by the free acid. It now turned the plane of polarisation still to *the left*, but to the extent of 3° 15′. Thus the rotation from 0 to the left had been much increased, more than doubled, by the introduction of the zinc and the attendant thermal and hydric operations.

The zinc salt, which had been obtained in a state of purity, as proved in the previous paragraph, was dissolved in water and decomposed with hydrothion; the free acid was concentrated and became a colourless syrupy liquid; in this state it was not perfectly brilliant, but had a slight haze, probably from a trace of finely divided sulphur. It was therefore allowed to stand for two months in a quiet place, and when the trace of particles had completely deposited, the clear part was isolated by decantation.

This acid, on being placed in a tube of 100 mm. in length into the polaristrobometer, now turned the plane of polarisation to *the right*, in the particular instance of an acid of uncertain concentration, to the extent of 2° 17′ (average of seven observations).

I have made no attempt to determine the specific rotating-power of the lactic-acid from the brain. The reason for this is the circumstance first observed by Wislicenus, that the rotating-power of sarkolactic acid changes under a great number of influences, such as heat, water, and time. Thus free sarkolactic acid, when dried over sulphuric acid in vacuo during 21 months, is transformed into a mixture of lactic acid, $C_3H_6O_3$ (16·50 per cent.); anhydride, $C_6H_{10}O_5$ (84·19 per cent.); and lactide, $C_3H_4O_2$ (16·04 per cent). The solution of this mixture turns the plane of polarised light to the left $(\alpha) = -85{\cdot}93°$. It is probable that all three products on treatment with water are transformed back into sarkolactic acid.

The watery solution of sarkolactic acid as ordinarily obtained, directly after extraction and concentration, shows a considerable polarisation to the left. This power is suddenly and greatly diminished after every addition of water or spirit, but on standing it rises again, without, however, reaching its former value. The diminution of specific rotatory power by dilution is the greater, the more concentrated was the solution used for dilution, that is to say, the greater was the dilution in proportion to the strength of the original solution. These changes are due to the presence of anhydrides and lactide. And as every preparation of sarkolactic acid contains these anhydrides, according to Wislicenus, pure sarkolactic acid, as a preparation, does not exist, and therefore its specific rotatory power, which this author surmised to be to the right, cannot be accurately determined.

I have shown above that pure sarkolactic acid prepared from the zinc salt turns the plane of polarised light freely *to the right.* But the solution of the zinc salt from which this acid was produced turned energetically *to the left.* The saturated normal solution of zinc salt turns the plane of polarised light steadily 7° 7′ to the left. In over-saturated solutions the turning faculty is not increased, as might be supposed, but, on the contrary, is diminished.

The polarising faculties of sarkolactic acid, its hydrate, zinc salt, and anhydride may be described as follows:

Turning farthest to the *right*	-	$C_3H_6O_3$.
Turning less far to the *right*	-	$C_3H_6O_3 + H_2O$.
Turning least to the *left* -	-	$2(C_3H_5O_3)Zn$.
Turning more to the *left* -	-	$2(C_3H_5O_3)Zn + 2H_2O$.
Turning farthest to the *left*	-	$C_6H_{10}O_5$.

Peculiarities of the Calcium Salt of Lactic Acid from Human Brain.

This salt was made from pure lactic acid obtained as above described. Its solubility in water was so great that attempts at its crystallisation from this solvent were foiled by the solution setting to a solid mass. It was consequently recrystallised from strong spirit. When dried in air, it was a light voluminous spongy mass of crystals, which on being dried at 105° lost 21·2 per cent. of water of crystallisation, and contained 14·14 per cent. of Ca.

These data do not correspond to any of the recorded data concerning this salt, which recorded data themselves differ from each other, or, on the assumption that there was only one hydrate, contradict each other. It is now assumed by some that the sarkolactate of calcium, as commonly obtained, has the formula—

$2Ca,4(C_3H_5O_3) + 9H_2O$ - - water	=	27·09 per cent.
Formerly, however, a hydrate was mostly described as containing water - -	=	24·83 per cent.
The hydrate just described contains water	=	21·2 per cent.

Now, the second salt corresponds to one with 4 molecles of water of crystallisation, while the last leads to no even proportion between salt and water of crystallisation, but is intermediate between the salt containing 4 molecles and a hypothetical salt containing 3 molecles of water, which requires 19·89 per cent.

The new salt must therefore be considered either as a compound or mixture in nearly molecular proportion of the salt, containing 24·83 per cent., with the hypothetical salt containing 19·89 per cent. of water, or, in more simple terms, as a salt consisting of 2 molecles of anhydrous lactate and 7 molecles of water of crystallisation, requiring 22·35 per cent. H_2O; possibly a lower homologue of the salt with 9 molecles of water. The depression of the water by mere admixture of anhydride was improbable, owing to the uniform character of the crystallisation.

I am, therefore, of opinion that there are at least three, if not four, different crystallised hydrates of calcic lactate, and that the

amount of hydration is probably dependent upon the concentration of the solution if it be a watery one, or upon the aquosity of the solvent if it be spirit.

The preparations of lactic acid from the brain of man and the ox, which I have described above, leave no room for doubt regarding their nature; they are specimens of the one optically active sarkolactic acid, yielding the precisely characteristic zinc salt; that they did not yield the ordinary calcium salt is of little consequence, as the question of the composition of the calcium salts of sarkolactic acid is not exhaustively answered. Whatever may be the issue of the discussion regarding the constitution of the different lactic acids, *the facts* now ascertained regarding cerebral lactic acid cannot thereby be affected.

I have disposed of some opinions regarding the alleged presence of a second acid in sarkolactic acid already in 1877 (compare my 'Pathology of the Urine,' 1877, p. 461 *et seq.*). I now prove for the brain, what Erlenmeyer has proved for the flesh, namely, that it contains only one lactic acid.

2. Formic Acid.

The acids extracted by ether after precipitation of the alkaloids were in one experiment placed in a retort, and subjected to distillation. The main part of the distillate consisted necessarily of acetic acid. The acids were neutralised by barium carbonate and evaporated to crystallisation; the first crystals gave 53·09 per cent. Ba (acetate requires 53·72 per cent. Ba); the second crystals gave 55·53 per cent. Ba, and gave a strong formiate reaction with nitrate of mercurous oxide. It is, therefore, probable that the water extract of the brain contains a small amount of *formic acid*, as has already been stated by Von Bibra and Müller. The amount was, perhaps, not greater than that of succinic acid, to be described.

3. Succinic Acid.

(*a*) *In Ox-brain.*—The mother-liquors of the zinc lactate gave with ferric chloride, not in the cold, but on boiling, a rust-coloured precipitate, soluble in excess of chloride, forming a dark-red solution. The united mother-liquors were cautiously precipitated while boiling, and the compound filtered off. They were next boiled with barium carbonate, and the precipitate filtered off.

The latter was extracted with hot dilute acetic acid, in which the ferric precipitate was insoluble. The ferric precipitates were dissolved in water, and H_2SO_4, and extracted with ether; the ether solution left the acid free.

The acid, easily soluble in water, crystallised on evaporation, fused, and sublimed in white vapours. The vapours had a pungent smell, and formed white crystals on condensation. The acid was entirely volatilised without leaving any charcoal.

The sublimated crystals dissolved in water, and gave a clear and colourless solution. This was cautiously neutralised by sodic carbonate. The solution gave a rusty precipitate with ferric chloride, soluble in excess of chloride. It gave a white precipitate with mercurous nitrate, not altered by boiling; gold chloride gave no reaction, which excludes malonic acid; uranic nitrate gave no reaction. After boiling and concentration the solution of the sodium salt gave a precipitate with $BaCl_2$. This was soluble in HNO_3, and reprecipitated by NH_4HO; still more by a little spirit.

This acid is consequently *succinic*, $C_4H_6O_4$.

(*b*) *In the Human Brain.*—The process described in the foregoing regarding the brain of the ox was repeated on the mother-liquor of zinc lactate from the brain of man, and exactly identical results were obtained. The ferric salt was decomposed and the acid extracted by ether. It remained in a crystalline state, and was sublimed. The reactions were then made upon the sublimate, and found to agree exactly with those of succinic acid.

This experiment was repeated on a second quantity with identical results.

Succinic acid is thus shown to be a normal ingredient in small quantity of the brain of man and of the ox. Müller, when extracting lactic acid from brain, had searched for succinic, but had not obtained any. This is explicable on several grounds: firstly, his method was not calculated to obtain it (he waited for crystals to form in the concentrated lactic acid); and, secondly, the quantities of brain-matter employed by him were probably too small.

The significance of succinic acid in nerve-marrow is probably connected with that of the disintegration of the albuminous substances. But the possibility of merely accidental presence must not be lost sight of, as succinic acid is present in many kinds and parts of vegetables used for food by man and animals, and in wine and other fermented liquids, in which it is produced by fermentation from sugar.

VI.

GROUP OF ALBUMINOUS PRINCIPLES.

The brain as a whole is an aggregated mass of bioplasm, which derives its peculiarity mainly from specific chemical additions. The latter have been treated of in the earlier parts of this treatise, and there remain for consideration the albuminous substances which constitute the stroma of the bioplasm, in which the specific matters are distributed, or with which they are combined in such a manner as to produce the living brain-tissue or neuroplasm. It is naturally deposited in the shape of *cells* and *fibres*, the cells being termed ganglionic, because they were first observed in nerve-ganglia, anatomically so-called. The fibres contain more of the specific principles than the cells, but after they are deducted, the albuminous principles in cells and fibres are very much alike. By analytical means some part of each of the principles can be extracted and identified, but it is as yet impossible to separate the whole of them from each other unchanged. It is found, principally by the method of extraction of the comminuted tissue by means of salt water of varying concentration, that neuroplasm contains small quantities of *soluble albumen*, partly exhibiting the properties of serum albumen; small quantities of *fibrin*, and considerable quantities of what, from its similarity to the body forming the stroma of the blood-corpuscles, has been termed globulin, but which by its function is characterised as a plastin. And in order not to assume that the plastin of the nerves is identical in every respect with the plastin of other organs, I propose to treat of it as *neuroplastin*. In the process of extraction of the five groups of constituents of the brain, the albuminous matters all become insoluble, and change their reactions with those agents in which they were previously soluble. The coagulated mass of brain-tissue after exhaustion with alcohol is

thus a mixture of curdled albumen, and of fibrin and neuroplastin changed by heat and the influence of alcohol. It contains, further, all the cytophosphatides from the nuclei of the nerve-cells, and from the sheaths of the nerve-fibres ; the material of these sheaths themselves, which is probably analogous in composition to that of the sheaths of the muscular fibres ; and the tissue of the capillaries, which pervade the brain-tissue. But neuroplastin is by far the greater portion of the insoluble residue, and albumen and fibrin do probably not amount to one-eighth of the weight of the neuroplastin. The total weight of the albuminous matters of the human brain, free from its membranes, amounts to 7·0 per cent. at least; in some parts it is 7·6 per cent., and may vary between these figures in different parts. Grey neuroplasm contains 7·6 per cent., white tissue 8·6 per cent. This is not quite equal to half the solids in grey neuroplasm, which amount to about 15 per cent., while in white neuroplasm the amount of solids rises much higher. The specific ingredients of white neuroplasm may amount to 19·16 per cent., and if 8·6 per cent albuminous matters are added to this, we have a total of 27·76 per cent. of solids, to which some salts have yet to be added. It is therefore not surprising that some specimens of white neuroplasm should yield as much as 30 per cent. of solids. The albuminous matters have sometimes been found as high as 10 per cent., but it is doubtful how far in these cases they were fully extracted with alcohol. As much valuable information regarding the albuminous substances in general had been obtained by chemolysis, even in cases where the substances could not be obtained in a pure state, I applied the process to neuroplastin in the first instance with the following results :

Chemolysis by Barita of Neuroplastin from Brain.

The albuminous matter was obtained by exhausting brain (human or ox) with spirit of 85 per cent. strength.

An amount of neuroplastin about 100 g. in weight, with six times its weight of crystallised barita and four times its weight of water (part of the water being used to soak the albumen for some time previous to the admixture of the barita), was taken for the chemolysis.

The apparatus employed was an autoclave of wrought-iron, and holding about 5 litres. The cover was air-tight and secured by a

screw clamp, while an adjustable valve provided for the escape of gas in case of the pressure rising beyond certain limits. The mixture above described was placed in the chemolyser, and the temperature raised to 180° C. by means of a gas-burner placed underneath. The heat was maintained at this height for six hours, whereupon the chemolyser was allowed to cool. When the autoclave was opened, its contents emitted a strong smell due to ammonia, compound ammonias, albuminol, and other products of decomposition.

The semi-solid mass was extracted, placed in a platinum-still with much water, and subjected to distillation. Two litres of distillate were drawn off, containing ammonia and compound ammonias in solution, and albuminol in small white flakes; the latter were removed by extraction with ether.

The ether solution on evaporation left a small residue consisting of *albuminol* mixed with a peculiarly smelling, probably *sulphurised body;* the latter was gradually volatilised on standing, leaving the albuminol, which crystallised.

The watery distillate was neutralised with hydrochloric acid and evaporated to dryness, and the mixture of salts was further treated for the separation of compound ammonias from simple ammonia, as will be described below.

The mixture from which the volatile alkalies had been distilled was filtered, and the insoluble matter isolated.

This insoluble matter was treated with water, ether, and hydrochloric acid. A small quantity of a fatty acid went into solution in the ether, barita with phosphoric and oxalic acid dissolved in the acid water, while barium sulphate remained insoluble.

The soluble matters filtered from the foregoing precipitate were freed from excess of caustic barita by crystallisation. From the filtrate all barita was removed by excess of sulphuric acid, and from the solution all acetic acid was expelled by distillation.

From the cold acid liquid *alkaloids* were removed by the addition of phospho-wolframic acid as long as a precipitate was produced. This precipitate was washed with water containing 5 per cent. of sulphuric acid, and then decomposed with barita. The solution containing the mixture of alkaloids was further treated, as will be described below.

The acid filtrate from the phospho-wolframates, containing *the amido-acids*, was freed from phospho-wolframic and sulphuric acid

by barita, and evaporated slowly to crystallisation. It deposited first tyrosin, then a mixture of leucin and tyrosin, then leucin and small quantities of other amido-acids. Then a syrupy mass remained, which contained yet some alkaloids, besides amido-acids. Then *alkaloids* were as far as possible removed by a repetition of the phospho-wolframic acid precipitation just described. From the liquid more leucin was obtained. At last there remained a small quantity of *uncrystallisable matter*, which was treated as follows:

It was precipitated by mercuric nitrate and sodium carbonate. The *bulky precipitate* was washed with water by decantation, etc., and decomposed by hydrothion. The solution on evaporation left a colourless gummy mass of the same character as the original matter. It will be further treated of below. The *mother-liquor* of the mercury precipitate was also freed from mercury, and found to contain but little organic matter.

The *mixtures of tyrosin and leucin* were united and warmed with water containing 10 per cent. of absolute alcohol. This dissolved leucin, and left tyrosin undissolved. The limits of accuracy of this process will be indicated lower down.

Tyrosin was purified by solution in hydrochloric acid, treatment with animal charcoal, and precipitation by sodium acetate; the precipitated tyrosin was crystallised from ammonia.

Leucin was purified by combination with copper, its solution being mixed and heated with copper acetate in a manner the details of which will be described below. In the course of this process a new leucin, the first isomer of the leucin hitherto known, was discovered, and from its sweet taste was named *glycoleucin.*

The copper-compounds of the leucins are almost insoluble in cold water, more soluble in boiling water. The copper-compounds of other amido-acids which accompany the leucins seem all to be more soluble in cold water, particularly those of lower atomic weights than leucin.

Glutaminic and asparaginic acid, which have been obtained by chemolysis from other albuminous substances, have not yet been isolated from the products of chemolysis of brain-albumen. It remains to be seen whether the compound alkaloids which have been observed will on long-continued chemolysis yield these acids. At present it seems as if the barita process applied to brain-

albumen did not produce them. It is, however, not impossible that they may be formed by the hydrochloric acid and tin process, which evolves them easily from casein.

Nitrogen as Ammonia from Neuroplastin.—Barita chemolysis of 10 g. and distillation gave volatile alkali, which was neutralised by hydrochloric acid, and combined with platinic chloride. The dry salt was redissolved, and left 0·0550 g. insoluble matter, while the soluble double salt of ammonio-platinic chloride amounted to 2·8760 g. This corresponds to 0·1803 g., or 1·803 per cent. N. The insoluble platinum salt contained 0·0077 g. N. This quantity of nitrogen is far below that obtained by the same process from other albuminous matters. It approaches that obtained from gelatin—namely, 2·55 per cent. It is less than one-fourth of the total quantity of nitrogen contained in the albuminous substance.

Total of Insoluble Barium Salts obtained from 10 *g. Neuroplastin.*—These salts dried at 110°, weighed 4·1760 g., and on analysis were found to have the following constituents:

Barium sulphate - - - -	0·083
Barium - - - - -	2·425
Phosphoric acid (PO_4) - - -	0·099
Oxalic acid - - - -	0·043
Sulphurous acid - - - -	0·008
Hydrothion - - - -	trace
Carbonic acid and soluble organic matter -	1·246
Organic insoluble matter - - -	0·272
Total - - - -	4·176

In the foregoing experiment about 1 per cent. of phosphoric acid was obtained.

Barium retained by Amido-Alkaloid-Acid Mixture.—The mixture of amido-acids, alkaloids, and acids from which all excess of caustic barita has been removed by carbonic acid, retains a considerable amount of barium in solution; this has to be removed previous to the distillation of the volatile acids. The amido mixture from 10 g. of albumen yielded 1·7830 g. barium sulphate, equal to 1·0480 g. Ba, or 10·48 per cent. This is only about half the quantity of barium retained in the chemolytic products of other albuminous substances, ossein excepted, in the products of which the barium retained amounts to 13·2 per cent.

Quantation of Acetic Acid from Neuroplastin.—The acid distillate from 10 g. of brain-albumen, chemolysed as above described, yielded 0·1586 g. barium salt ($BaC_4H_6O_4$, At. W. = 255) equal to 0·074 acetic acid, or 0·74 per cent. This is only one-fourth of the quantity yielded by pure albumen, and only half that yielded by ossein or ichthyocollin.

Quantation of Tyrosin from Neuroplastin.—The tyrosin obtained by the chemolysis of 10 g. of substance was separated by alcohol, recrystallised from ammonia, and dried at 105°. It weighed 0·122 g. = 1·22 per cent. This is the smallest percentage of tyrosin as yet obtained from any albuminous substance by this process. Tyrosin is retained by the amido-mixture, and even by crystallised leucin. The quantities of tyrosin actually isolated must therefore be considered as minima until absolutely reliable methods for its isolation may have been found out.

Leucins from 100 *g. of Ox-Neuroplastin.*—They were combined with copper by solution in boiling water and addition of excess of copper acetate. On boiling, a precipitate ensued, which was filtered off and washed with cold water until the washings were no longer blue. This precipitate was now boiled with much water, and the blue solution was evaporated to dryness. The residue from this evaporation is product No. 2, while the salt which remained insoluble in boiling water (*i.e.*, in as much as was applied) constitutes product No. 1. The mother-liquor of the first precipitate was evaporated as long as precipitates were formed. These constitute product No. 3.

Quantation of Copper in the three Products.

No. 1	contained - - -	19·05 per cent. Cu.
No. 2	„ - - -	20·26 per cent. Cu.
No. 3	„ - - -	19·5 per cent. Cu.

The latter amount of copper is almost that required by cupric dileucin. The solution obtained by decoction of the first precipitate contained probably some amido-acid of lower atomic weight, which raised the amount of copper to 20·26 per cent.; while the residual salt probably contained an amido-acid of higher atomic weight than leucin, whereby the amount of copper was depressed to 19·05 per cent. These hypotheses will have to be further tested on larger quantities of material.

Organic Body from Mercuric Chloride and Soda Precipitate.

The white precipitate was decomposed with hydrothion, and the solution left a semicrystalline substance. This was insoluble in spirit of 85 per cent. strength. In its watery solution phospho-wolframic acid produced no precipitate, showing that it was not and did not contain any alkaloid. Alkaline copper solution on application of heat gave no reaction. Ferric chloride produced a brown precipitate, soluble in excess of chloride, with an intensely red colour. Ammoniacal silver solution gave no precipitate, but on boiling it deposited reduced silver. Copper acetate gave no precipitate even on addition of alcohol. Acetate of lead gave a precipitate only after the addition of much alcohol, and this compound seemed to be an alcoholate. When dry it was found to contain 57·8 per cent. of lead. It was therefore probable that the body was not and did not contain either glutaminic or asparaginic acid.

Properties of Common Tasteless Leucin and of its Copper-compound, studied with a view of establishing its Diagnosis from its Isomer Glycoleucin.

Some tasteless leucin, made from albuminous matters (not brain) by the sulphuric acid process, was treated with cupric acetate, and the first precipitate was removed. (In this any glycoleucin would have been contained.) The filtrate from the first precipitate on evaporation gave a precipitate (termed 'first precipitate by evaporation,' being second precipitate of copper process), which was decomposed by hydrothion. The solution, on concentration, gave a first crop of crystals, which were rhombic plates, and *quite tasteless.*

This was again combined with copper, and the precipitate was filtered off. The precipitate was now boiled with water repeatedly until it was completely dissolved, and the solution was filtered hot.

Quantations of Solubility.

(*a.*) 730 cc. saturated boiling deposited, mainly immediately after filtration, and only to a small extent during cooling and standing for twenty-eight hours, blue light crystals, which when dry, weighed 0·1790 g., indicating a deposit of 1 part out of 4,078 parts of boiling water.

(*b.*) Of the clear blue filtrate from the foregoing operation 630 cc. were evaporated to dryness, and left 0·1048 g. of blue compound, showing that 1 part was soluble in 6,011 parts of cold water.

(*c.*) 100 cc. on evaporation left 0·0162 g. residue, equal to a solubility of 1 part in 6,172 parts water.

Results: 730 cc. of the leucin copper solution contained while saturated at the boiling heat 0·3000 g. of salt; of this, 0·179 g. was deposited on cooling, while 0·1210 g. remained dissolved at the ordinary temperature. The solubility of the salt in boiling water is therefore 1 part in 2,433. This is about double the solubility of glycoleucin copper.

It is generally stated in chemical writings that leucin dissolves in 27 parts of water at the ordinary temperature. I find by special experiment made with pure tasteless leucin, that 1 part dissolves in 30 parts of water at 15° C. (Of glycoleucin, 1 part is soluble in 82 parts of water at 18° C.) It requires 658 parts of spirit of 75 per cent. strength for solution. Combination with copper therefore reduces its solubility very greatly.

The immediate deposition of crystals from a saturated leucin-copper solution filtered hot indicates the presence in the compound of ordinary or tasteless leucin; the hot saturated solution of glycoleucin-copper differs from the former in this, that it forms a deposit only after long standing.

Stability and Regularity of Composition of Leucin Copper Compound.

A quantity (2·3 g.) of crystallised ordinary tasteless leucin, of similar origin as the foregoing, and separated from copper with which it had been combined, was a second time converted into the copper compound by means of copper acetate. The compound was extracted several times with boiling water, and then dissolved entirely in a sufficiency of boiling water. Soon after filtration there separated from the hot solution *a crystalline deposit*, which increased only slightly while the liquid cooled to the ordinary temperature of the air. The precipitate was isolated and dried at 110°, and contained 19·15 per cent. Cu. The solubility of this precipitate in boiling water was as follows: 200 cc. of boiling solution saturated by long boiling with excess of salt left 0·0904 residue, equal to a solubility of 1 part in 2,212 parts

of boiling water. The cold solution filtered from the precipitate on evaporation left a residue, which showed that 1 part had been dissolved in 5,882 parts of water.

The *solution* in which the crystalline deposit, described in the foregoing, had been formed, was evaporated to a small bulk as long as it formed any deposit while hot, and then allowed to cool. The isolated (here termed 'second') precipitate dried at 110°, contained 19·57 per cent. Cu. 200 g. of cold water dissolved 0·0432 g. of this compound, which is equal to a solubility of 1 part in 4,630 parts of water.

The filtrate from the second deposit just described was evaporated to dryness to obtain the remainder of the leucin compound. Some copper compound, differing in appearance from the leucin compound, was deposited *with* the latter, and had to be removed by water acidulated with a little acetic acid. This, as the quantation of the copper contained in the compound showed, succeeded only partially, for it still contained 20·39 per cent. Cu.

Thus it will be seen that the very first crystals of this preparation (reconstituted a second time as copper-salt, after having been constituted the first time as copper salt, with an apparently pure specimen of leucin) contained a little less copper than is demanded by theory; the second crystals, obtained by concentration, yielded the theoretical amount of copper; and the third fraction was evidently impure by the admixture of a copper salt, which was perhaps a combination of an amido-acid of lower atomic weight than leucin.

In connection with this it deserves to be mentioned that the presence in leucin of tyrosin as an impurity depresses the amount of copper which will be found in the copper compound made from such leucin. 11·2 g. of crystallised leucin were treated in boiling watery solution with copper acetate, and the deposit was collected. It gave on analysis (mean of two quantations) 17·25 per cent. Cu. The filtrate on further evaporation and cooling gave another deposit, which on analysis was shown to contain 18·18 per cent. Cu. Another 11·2 g. of the same leucin as that used in the experiment just related, was dissolved in a large quantity of warm water, and allowed to cool slowly, when it deposited a sensible portion of *tyrosin.* The leucin was now removed by the copper process, and the compound found to yield the theoretical amount of copper. The mother-liquors of the first and the

second part contained a copper salt of a lower amido-acid, which had a sweet taste, but was not glycoleucin.

Note.—This and similar copper salts, not yet exactly identified, retain tyrosin in solution. When the copper is now removed by hydrothion, the filtrate, reduced to the same bulk as the original solution, deposits *tyrosin.* This first deposited tyrosin gives a good mercurous nitrite test, but may contain some of the following *new body.* When the mother-liquor of the foregoing tyrosin is slightly evaporated, it deposits a body much like tyrosin, crystallising in microscopic fine needles, but giving no mercurous nitrite reaction ; a slight rose-pink which appears at the moment when the reagents are mixed, disappears, and the mixture becomes white.

Leucin obtained by Crystallisation from Amido-Mixture from Chemolysis of Neuroplastin.—Although this leucin had no distinctly sweet taste, it was treated with copper acetate to separate any glycoleucin which might be contained in it. It was dissolved in hot water and heated to boiling ; a concentrated solution of copper acetate was now added gradually until a precipitate was formed. This *first precipitate* was removed by the filter. To the filtrate was added copper acetate as long as a precipitate was produced, and then a slight excess of the acetate. The mixture was allowed to stand and deposit the *second precipitate.* This was collected on a filter. The *filtrate* was evaporated to a small bulk, and deposited, particularly on cooling, a *third product.* This latter appeared the most crystalline, and of the deepest blue colour.

The first precipitate was then boiled seven successive times with large quantities of water to extract the common leucin copper compound, and leave any glycoleucin copper compound insoluble behind. It was then dried on the filter ; lastly in the air-bath at 110°, and analysed ; it contained 19·60 per cent. Cu, or exactly the theoretical amount of cupric dileucin.

The second precipitate was boiled with nine successive large volumes of water, and what remained undissolved was analysed ; it contained 19·49 per cent. Cu.

The third precipitate which crystallised out of the concentrated mother-liquor of the first and second precipitate, was finely triturated in a mortar, washed with cold water, dried at 110°, and analysed ; it contained 19·60 per cent. Cu (again exactly the theoretical amount required by cupric dileucin).

The first and second precipitate were united, suspended in boiling water, and decomposed by a current of hydrothion. The bulk of the copper-sulphide was removed by filtration; the hazy and coloured liquid was evaporated to collect and precipitate some copper-sulphide (which is frequently imperfectly precipitated from neutral organic liquids), and after final filtration evaporated to crystallisation. The latter process was aided by the addition of some strong alcohol. The crystals were collected, and recrystallised from water and alcohol and dried in the air-bath. On elementary analysis they yielded results agreeing closely with the theory of pure leucin.

Glycoleucin, the first Chemolytic Isomer of Leucin, its Properties and Combinations.

Glycoleucin has been obtained by me in two ways, namely, synthetically, and by the chemolysis with barita of brain albumens. I have not obtained it from animal albuminous matters by the sulphuric-acid process, which yields ordinary tasteless leucin.

The mode in which it is separated is the following: The mixture of the several leucins, obtained by crystallisation from the amido-mixture, is combined with copper, by treatment with acetate. The glycoleucin copper compound is mainly deposited with the first part of the precipitate; later, a mixture is deposited; lastly, mainly the compound of ordinary leucin. The copper compounds which result are then exhausted with boiling water. The glycoleucin copper compound, as the least soluble, remains behind, and is ultimately almost insoluble in boiling water. Much glycoleucin copper of course dissolves with the compound of ordinary leucin. The latter can to a large extent be isolated by the property that it separates from the boiling saturated solution in water almost immediately after filtration, while the saturated boiling glycoleucin copper solution does not deposit the excess of its salt until after long standing. It will be evident that these processes can only yield small quantities of material at a time. Seven specimens of glycoleucin copper were decomposed by hydrothion, etc., and crystallised separately. Three of the crystallised products were subjected to elementary analysis, and showed the identity of their composition with that of leucin. The seven specimens of crystals were then united (they weighed 7·53 g.) and dissolved in boiling water. To the boiling solution

a concentrated cold solution of cupric acetate was added, not in excess. A sky-blue precipitate ensued, and was filtered off while the liquid was hot. To the filtrate, while cooling, a further quantity of cupric acetate was given, until no further precipitate was produced.

Precipitate No. 1.—Dried at 110° contained 19·20 per cent. Cu.
Precipitate No. 2.—Dried at 110° contained 19·45 per cent. Cu.

An eighth specimen of pure glycoleucin was combined with copper, and the compound on analysis gave 19·45 per cent. of Cu. The mother-liquor of this on concentration yielded a preparation which on analysis was found to contain 19·42 per cent. Cu.

These data agree pretty closely with the theory of monocupric dileucin, which contains 19·60 per cent. Cu = $2(C_6H_{12}NO_2)Cu$, or $C_{12}H_{24}CuN_2O_4$.

Solubility of the Cupric Salt of Glycoleucin in Cold Water.—Pure salt was boiled for a long time with water; the mixture was allowed to cool; when quite cold the solution was filtered from the compound. 200 cc. at 16·5° C. on evaporation left 0·0228 g., equal to a solubility of 1 part in 8,772 parts of water. The solubility of ordinary leucin copper is 1 part in 6,172 parts of water, and is therefore greater than that of glycoleucin copper.

During the operation no trace of reduction or blackening was observed, not even when the solution of the compound was evaporated to an extremely small bulk with excess of cupric acetate. (This constitutes a diagnostic difference from the bearing of a more soluble sweet product which is obtained from the mother-liquors of the amido-mixture, and during the evaporation of which with copper acetate such a reduction takes place.)

Solubility of the Cupric Salt of Glycoleucin in Boiling Water.—200 cc. of the boiling solution left on evaporation to dryness 0·0449 g., or 1 part dissolved in 4,454 parts of boiling water. This is about double the solubility of the salt in cold water. On cooling, the solution becomes only slightly opaque; a long time is required for it to deposit the salt soluble in the hot as a visible precipitate.

The glycoleucin copper compound crystallises in minute scales and plates, combined in masses or balls. Many of the scales are clearly rhombic, others rhombo-hexagonal.

Elementary Quantation of Glycoleucin, prepared from Insoluble Copper Salt.—The copper was removed by hydrothion, and the

glycoleucin obtained pure and pearl-white by repeated crystallisation. It was dried over calcium chloride, and at last at 110° C.

Synopsis of Theory and Data.

At.	Wgts.	Percent.	Found. (*a.*)	(*b.*)	(*c.*)
6 C	72	54·96	54·92	—	—
13 H	13	9·92	10·02	—	—
N	14	10·69	—	10·61	10·72
2 O	32	24·43	—	—	—
	131	0·00			

Glycoleucin does not give the inosite reaction with mercuric nitrate.

Solubility in Water at 18°.—100 parts by weight of solution retain 1·22 parts of glycoleucin in solution, or in round numbers 1 part is soluble in 82 parts of water. Glycoleucin is therefore much less soluble in water than common tasteless leucin, of which 1 part requires 30 parts of water for solution at 15°.

The sweet taste of glycoleucin is less easily perceived on the crystals than on their saturated solution. Of this one drop will give a distinctly sweet taste over a great part of the mouth. The intensity of the sweetness is not much less than that of inosite.

A New Reaction and Compound of Cerebral Tyrosin.

I have shown that tyrosin is present amongst the amidated bodies extracted from the brain, though in small quantities only. It is also a product of the chemolysis of neuroplastin, and cannot be separated so completely from the amido-mixture as is desirable, and as is generally believed. To improve the processes of this separation I have made some experiments which have resulted in the following. The experiments were made on tyrosin from neuroplastin.

Tyrosin dissolved in hot water with the aid of caustic soda ley, on addition of mercuric chloride, gives a deep yellow solution, and no precipitate. On cooling the mixture becomes turbid, and on reheating a yellow precipitate ensues. The solution of tyrosin must be *dilute*, *hot*, must contain excess of caustic ley, and the mercuric chloride must not be added in excess; if excess be added, a yellow precipitate is immediately produced. If to the dilute, hot, alkaline solution of tyrosin mercuric chloride is added

gradually, until a faint yellow precipitate is produced, and the solution is heated and allowed to stand, the slight precipitate which forms on heating crystallises entirely in golden-brown crystals, and is Hg_2OCl_2. The yellow amorphous precipitate, which forms out of the yellow solution on application of heat, is entirely soluble in excess of caustic soda. On heating the solution to boiling, no immediate precipitate is now produced. If the heating and addition of mercuric chloride are done very gradually near the boiling-point, until the yellow fluid becomes opaque, it sets on cooling into a white solid jelly. This compound is dissolved in excess of caustic soda, the solution is filtered, and the compound is then reprecipitated with acetic acid. It is then perfectly white. The compound is therefore insoluble in weak soda, soluble in great excess of soda, and insoluble in dilute acetic acid. Dried over oil of vitriol in vacuo and analysed, the compound gave the following data :

Synopsis of Data and Theory.

	Found Percents.	÷ by At. Wgts.	÷ by N = 1.	Hypothetical Formula.
C	18·25	1·520	8·7	C_9
H	1·78	1·780	10·3	H_{10}
N	2·42	0·1728	1·	N
Hg	61·37	0·3069	1·77	Hg
Cl	6·48	0·1825	1·	Cl
O	9·70	0·6062	3·4	O_3
	100·00			

$$\left.\begin{matrix} C_9 \\ H_{10} \\ N \\ Hg \\ Cl \\ O_3 \end{matrix}\right\} \times 2 + HgO.$$

or $2(C_9H_{10}NO_3 + HgCl) + HgO$, or expanded in the following formula :

$$\begin{matrix} Hg-Cl \\ | \\ \left.\begin{matrix} C_9H_{10}NO_3 \\ | \\ C_9H_{10}NO_3 \end{matrix}\right\} HgO. \\ | \\ Hg-Cl \end{matrix}$$

It will thus be seen that the reaction essentially consists in a reduction of mercuric to mercurous chloride, which remains combined with tyrosin ; and of this compound two molecles are soldered together by a molecle of mercuric oxide. It is not intended to fix the exact manner in which, and place (in the molecle) at which this union is effected. The reduction of the

mercuric to mercurous chloride is no doubt effected by that part of the hydrogen of the tyrosin which does not appear in the precipitate; in other words, an atom of hydrogen is substituted by calomel. For this latter substitution the ordinary didynamic character of mercury affords an easy opportunity, but for the soldering action of mercuric oxide the metal may hypothetically be allowed to possess a greater number of dynamicities than two.

Mercuric chloride is a general reagent for bodies of the alkaloidal class. In this capacity it produces precipitates in almost all animal fluids which contain albuminous matters or their derivates down to ammonia. The composition of these precipitates is as yet little studied, but is complicated enough to have deterred inquirers from ascertaining it. I have examined some such precipitates, *e.g.* from the extract of the liver in kidney diseases. In such, the reduction of mercuric to mercurous chloride is effected massively, but only a part of the calomel produced remains in combination. The foregoing is a key to the study of such combinations.

New Alkaloids obtained from Neuroplastin by Chemolysis.

The amido-mixture, which is obtained from neuroplastin by decomposition with barita under pressure, yields on first crystallisation leucin, glycoleucin, tyrosin, and some amido-acids of lower atomic weight than leucin. But there remains a considerable part of the mixture which does not crystallise in the state of mixture, each ingredient of which, however, seems crystalline after complete separation. I have isolated two of the ingredients, and give in the following a short description of their isolation and their properties.

Mode of Isolation of Alkaloids from Amido-Mixture.—The mother-liquor of leucin is diluted, acidified with sulphuric acid, and mixed with an acidified solution of phospho-wolframic (syn. phospho-tungstic) acid, as long as a precipitate is produced. The latter is washed with water containing 5 per cent. of oil of vitriol, by decantation, ultimately on the filter. It is then decomposed with hot barita solution, the excess of barita is removed by carbonic acid, and the resulting solution of alkaloids is evaporated to a syrup. This product has the following properties and reactions: It is strongly alkaline; has the smell of sperma; is soluble in

ammonia water; does not reduce potassio-cupric tartrate. With hydrochloric acid and gold chloride it gives a copious precipitate, which is insoluble in excess of acid, easily soluble in alcohol. Hydrochloric acid and platinic chloride give no precipitate in watery solution, but in alcohol a precipitate is produced. Zinc chloride gives a copious white precipitate, which is soluble in excess of the chloride and in hydrochloric acid. Silver nitrate gives a voluminous white precipitate, soluble in nitric acid. Tannin gives a voluminous white precipitate. Mercuric chloride produces a striking phenomenon. When to its saturated solution a drop of alkaloidal matter is added, the whole surface is instantly covered with a white precipitate. (The matter precipitated is therefore not spermatin, which gives only a turbidity with mercuric chloride. Ann. Chem. Med. i. 306.) The alkaloidal matter is carbonated, as on addition of an acid carbonic acid is evolved. It also contains some barium not precipitable by carbonic, precipitable by diluted sulphuric acid.

Separation of the Alkaloidal Matter into two Groups by Absolute Alcohol.—When the syrupy mixture of alkaloids is treated with absolute alcohol, a viscous matter remains insoluble, another portion dissolves in the alcohol. The matter insoluble in alcohol retains its solubility in water. The matter soluble in alcohol gives with platinic chloride a copious precipitate of a double salt. This is not changed by alcohol, but is altered quickly by water. It fuses after evaporation of the alcohol. Placed in water it practically dissolves, a part remains undissolved. The solution in water continues to form deposits, for weeks, of the insoluble salt. Owing to this lability I have not examined these two platinum salts any further.

From the viscous matter precipitated by alcohol there was obtained by a process of continued crystallisation a white crystallised *alkaloid*, very soluble in water, easily soluble in hydrochloric acid. The hydrochlorate evaporated to dryness, and redissolved in water, crystallises from concentrated solution. From spirit this hydrochlorate crystallises still better. The spirit solution may be mixed with ether without yielding a precipitate. But the crystals of the hydrochlorate are not very soluble in ether and may be washed with it. The salt, mixed with mercuric chloride and then with caustic soda, gives a white precipitate soluble in excess of the soda.

Elementary Quantation of this new Alkaloid.—After removal of the hydrochloric acid the free body crystallises in balls of needles. Dry at 110°.

The analyses lead to the following provisional theory :

Found	Percents.	÷At. Wts.	÷N = 1.
C	52·99	4·4158	6·3
H	9·03	9·03	12·9
N	9·896	0·7068	1·
O	28·084	1·755	2·5
	100·000		

These relations of elements remind of a *leucein* (rather than a leucin), with which probably a small quantity of a higher homologue is mixed.

Separation of the Alkaloidal Matter into two Groups by Cupric Acetate and Absolute Alcohol.

When the mixture of alkaloids is dissolved in little water and mixed with a saturated solution of cupric acetate and warmed, no precipitate of leucin copper ensues if the process of precipitation by the phosphotungstic acid has been correctly conducted. On continued warming some brown cuprous and dark cupric oxide are deposited. The deep blue solution filtered from all deposits is mixed with absolute alcohol as long as this agent produces a precipitate. This is the copper compound of a new alkaloid, which, without further purification, on analysis gave the following preliminary results :

Synopsis of Result and Theory.

	Percents.	÷At Wgts.	÷Cu = 1.
C	38·23	3·186	12·95
H	5·91	5·91	24·
Cu	15·60	0·246	1·
N	10·61	0·757	3·
O	29·65	1·853	7·53
	100·00		

Leading to formula $C_{12}H_{23}CuN_3O_7$, or $C_{12}H_{23}N_3O_6 + CuO$. The salt has a very light blue colour ; it is easily soluble in water, and the solution has a very dark blue colour.

The alkaloids which are soluble in absolute alcohol in the presence of cupric acetate have not yet been isolated. They contain, of course, the bodies which give the platinum compounds above described. I have subjected them to numerous reactions, and have isolated some peculiar bodies. But they must be produced in quantity before they can be studied with advantage.

These alkaloids were not observed by Schützenberger in the researches on the chemolysis of various albuminous substances of which I have given an abstract in Ann. Chem. Med. i., pp. 20-44. They will, therefore, have to be added to the list of terminal cleavage products.

VII.

GROUP OF INORGANIC PRINCIPLES, OR MINERAL ACIDS, BASES, AND SALTS.

The ash of the fresh grey tissue of man amounts to about 1 per cent. ; that of white tissue is about 1·7 per cent. ; the same relations are observed in the grey and white tissues of the ox. From the former data it is apparent that an ordinary human brain contains from 18 to 20 g. of ash or incombustible residue. This residue consists, however, to the extent of about 48 per cent. of phosphoric acid, of which about one-fifth is in the free state, while four-fifths are in combination. The greater part of this phosphoric acid is unquestionably derived from the phosphatides, and has replaced nearly all the more volatile acids, such as carbonic, hydrochloric, and sulphuric acid. But even so the phosphoric acid included in the 1·7 per cent. of incombustible matter is certainly less than one-third of the phosphoric acid which is present in the phosphatides. It is therefore clear that a great portion of the phosphorus present in brain is volatilised in the course of the ordinary method of combustion. This method, therefore, actually leaves only the bases in an approximately complete manner. 100 parts of acid brain-ash contain 32 parts of potash, 11 parts of soda, and some lime and magnesia, for which we do not give figures, because we have none of our own, but know that those given by others are in correct. The analysis of the mineral ingredients of the brain will therefore have to be resumed in the manner indicated in the chapters which treat of the purification of the phosphatides, the cerebrosides, and the final water-extracts. These latter, we know already, contain sodium chloride, and sulphates, and phosphates, besides salts of organic acids. The cerebrosides and the

phosphatides remaining mixed with them have a great tendency to retain potash, in a form which is not precisely known. The phosphatides retain salts and oxides, amongst them lime, as shown in a previous chapter. Future analyses for the mineral salts will therefore have to be applied to four different materials, the neuroplastin, the phosphatides, cerebrosides and cerebrinacides, and the ultimate water-extracts.

VIII.

QUANTITATIVE RELATIONS OF THE IMMEDIATE PRINCIPLES AND CONSTITUENTS OF THE BRAIN.

A. QUANTATION OF THE CHEMICAL CONSTITUENTS OF GREY TISSUE FROM THE HUMAN BRAIN.

Grey tissue was carefully cut from the surface of both hemispheres, and from the anterior and posterior lobes of each. 6·2395 g. were dried at 95° until the weight was constant. The substance was repeatedly cut up with a knife. The dry residue weighed 0·9193 g. = 14·73 per cent. The water lost amounted to 5·3202 g. = 85·27 per cent.

For the following operations another quantity of grey tissue, amounting to 46 g. fresh, was cut from all parts of the brain. It was extracted five times with boiling spirit in a flask attached to a reflux cooler.

The *albuminous residue*, dry, weighed 3·5 g., equal to 7·6 per cent. of the fresh grey tissue. It was analysed for sulphur and phosphorus. 1·5304 g. were burnt with addition of barita-water, and gave 0·0024 $BaSO_4$ = 0·02 per cent. sulphur, and 0·0325 magnesium pyrophosphate = 0·60 per cent. phosphorus.

The *deposit* which the spirit made on cooling (cerebrins, etc.) weighed 0·3 g.

The *spirit solution*, evaporated to half its bulk, gave a (second) deposit which weighed 0·1 g.

The *filtrate* from this was evaporated to a small volume, and deposited a (third) deposit weighing 0·7 g.

The foregoing deposits (0·3 + 0·1 + 0·7, total = 1·1 g.) were extracted with ether. There remained 0·2 insoluble, 0·9 were soluble in ether. The matter soluble in ether (previously deposited from spirit) amounted to 1·950 per cent. of the fresh

tissue, while the matter insoluble in ether (cerebrins mainly) amounts to only 0·434 per cent. of the tissue.

The *last oily matter*, which could not be filtered from the water-extract in which it was suspended, was precipitated with lead acetate; the curded precipitate was collected on a filter.

The *precipitate of lead salts* contained the phosphorised organic compounds and sulphuric and phosphoric acid from the inorganic salts. It was washed while being continuously stirred with water to which a little lead acetate had been added. It was next extracted with absolute alcohol. The alcoholic solution contained all the *lecithin* (with some lead). The dry matter weighed 0·73 g.

The insoluble in alcohol residue was completely dried and extracted with ether. The solution on distillation left all *kephalin lead*, which weighed 0·22 g.

The insoluble in ether residue was extracted with boiling alcohol, which dissolved matters which when dry weighed 0·03 g.

The residue from the three extractions with alcohol, ether, and boiling alcohol was mainly an inorganic lead salt. It was exhausted with dilute nitric acid, and the insoluble residue was recognised as *lead sulphate*. The matter soluble in nitric acid was analysed for phosphorus by ammonium molybdate, etc., and gave 0·0049 P. The *phosphoric acid* thus indicated was evidently combined with alkalies in the grey tissue.

The lead salts from the mixture of the last oily matter and watery extract obtained as just described are liable to contain some *inosite lead* as an insoluble compound. When this is decomposed in warm absolute alcohol by hydrothion, an alcoholic solution is obtained from which, on long standing, and again after concentration, *inosite* is deposited in crystals. I have carefully identified these crystals, by their taste, their shape, and their reaction with mercuric nitrate. It is therefore clear that inosite can be precipitated, under the conditions prevailing in this research, by *neutral acetate* at least in part. Its complete precipitation can be effected only by *basic acetate*, and that with the aid of ammonia.

The watery solution was freed from lead by hydrothion, condensed to expel acetic acid, acidified with sulphuric acid, and extracted with ether. The extract gave by the usual treatment *zinc lactate* in crystals, which weighed 0·07 g., equal to 0·0452 g.

lactic acid in 44 g. grey tissue, or 0·102 per cent.; in round numbers, one part of lactic acid in a thousand of grey tissue.

The solution from which lactic acid had been extracted was treated with ammonium carbonate in excess, and gave clear indication of the presence of *calcium.* The filtered solution was boiled to expel ammonia, and then treated with phosphomolybdic acid. A precipitate of *alkaloids* or extractives was obtained weighing 0·530 g.

From the filtrate all phosphomolybdic and sulphuric acid were removed by barita. The filtrate treated in the usual way and evaporated to dryness left a residue, being *inosite and carbonates of alkalies* weighing 0·69 g. It gave with mercuric nitrate the inosite reaction. The residue was dissolved in water, and precipitated by lead acetate and ammonia. The precipitated inosite lead was isolated and decomposed by hydrothion.

The mother-liquor of inosite lead was freed from lead, evaporated, burnt, etc., and left mixed carbonates of alkalies, which were transformed into chlorides = 0·2560. Out of this quantity platinic chloride precipitated 0·070 double chloride = 0·0112 K = 0·0250 per cent. K in grey tissue. The remaining sodium amounted to 0·0920 per cent. of grey tissue.

Synopsis of the Results of Analysis of Grey Tissue of Human Brain.

	Percents.
Water expelled at 95° - - -	85·270
Neuroplastin - - - -	7·608
Ether extracts, kephalin, and lecithin (and cholesterin ?) - - -	1·950
Cerebrins and myelin - - -	0·434
Lecithin, Kephalin, Myelin } from last oily (and cholesterin ?) - - -	0·780
Inosite - - - - -	0·193
Lactic acid - - - -	0·102
Alkaloids - - - -	—
Sulphuric acid - - - -	traces
Phosphoric acid, H_3PO_4 - - -	0·017
Potassium - - - -	0·025
Sodium - - - - -	0·092
Water-extract - - - -	0·500
Loss in operations - - -	—

The loss in operations is very large, amounting to almost a quarter of the entire solids. It is mainly incurred through the difficulty which there is of separating the last oily matter from

the matter soluble in water. But other operations equally involve as yet unavoidable loss. The construction of suitable apparatus for ether extraction would only partly avoid the loss by ether extraction, as in this process the main difficulty is filtration.

B. QUANTATION OF THE CHEMICAL CONSTITUENTS OF THE WHITE TISSUE OF THE HUMAN BRAIN.

The parts to be analysed, amounting to 66 g., were cut from the centre of the hemispheres and corpus callosum. They were triturated to a pulp, mixed with spirit, and extracted six times with boiling spirit.

The *spirit extract* deposited a white *cerebrin substance* on cooling, which was collected and washed. It was then extracted with large volumes of ether and filtered again. It weighed, dried at 70°, 4·5615 g., equal to 6·91 per cent. of the fresh white brain-substance. Further treatment of the cerebrin bodies see below.

Ether extract of the cerebrin deposit.—The ether was distilled off, the concentrated solution was filtered from a trifling deposit, and distilled to dryness. The residue weighed 2·4150 g.

The ether extract of the albuminous substance (made after exhaustion by spirit and drying) on concentration deposited a few oily drops, and on distillation to dryness left a residue weighing 0·0350 g. It became dark, and existed only as a minute brown coating on the glass, possibly a mere trace of phosphorised substance, too small in quantity for further treatment.

The neuroplastin residue was dried and weighed, after the treatment with ether, 5·70 g., equal to 8·63 per cent. of original white brain-substance.

The *spirit extract* which had deposited the white matter was concentrated twice in succession, and after each concentration deposited a semi-crystalline buttery matter. Of this the ether extract weighed 5·1730 g., while the part insoluble in ether weighed 0·1400 g. Of this a part was soluble in boiling spirit, and deposited on cooling, while a dark part was insoluble in boiling spirit, and contained some inorganic matter and phosphorus.

The *cerebrin mixture* above described, weighing 4·5615 g., was dissolved in boiling absolute alcohol, when 0·1815 g. of coloured matter, which was insoluble in benzol, and therefore was not stearoconote, remained undissolved, =0·275 per cent. of tissue. (It amounted to 3·9 per cent. of the cerebrin mixture, and besides some slight impurity was neuroplastin.)

The dissolved part deposited, on cooling and standing, phrenosin, kerasin, cerebrinic acid, with some phosphorised matter, and retained a phosphorised matter in solution. The dry cerebrins weighed together 2·6030 (equal to 3·94, in round numbers 4 per cent., of the white tissue).

The alcoholic solution from which these cerebrins had been deposited, measuring 750 cc., contained 1·770 g. of matter dissolved, being phosphatides, amidolipotides, cerebrosides and cerebrinacides. With this some attempts at identification and quantation were made. Out of one half, 375 cc., a precipitate was obtained with the aid of alcoholic platinic chloride; the precipitate, dried at 65°, weighed 0·4524 g., and contained phosphorus = 2·52 per cent. The second 375 cc. were precipitated with alcoholic cadmic chloride; the precipitate weighed =0·2965, and contained phosphorus = 4·78 per cent.

The cadmium salt therefore contained much more phosphorus than the platinum salt; in other words, they contained different organic ingredients. Moreover, the precipitants brought down only about one-sixth part of the matter in solution in absolute alcohol. The mother-liquors were concentrated and made deposits, which were examined, but the results were not of a nature to be quoted.

The substances which remain dissolved in much absolute alcohol amount to 2·6 per cent. of the white tissue.

The last oily matter which was suspended in the aqueous extract could not be separated by filtration. This is one of the greatest difficulties of brain analysis, and will have to be overcome by further discovery. In the interval I have adopted the following process, which is efficient in all directions except on the point of inosite, as I have already stated above. The mixture was treated with lead acetate; the precipitate was dried (and weighed 0·7340 g.). It was extracted with cold absolute alcohol. (The extract weighed 0·4802 g.) It was mainly *lecithin*, with some lead.

The part insoluble in cold absolute alcohol was extracted with ether; the solution left a residue of *kephalin lead*, weighing 0·2030 g.

The part insoluble in ether was mainly *myelin lead*, but contained some sulphuric and phosphoric acid. It weighed =0·1970 g.

The watery filtrate from the lead precipitate was freed from lead by hydrothion evaporated to dryness, and left a residue 0·9260 g. This was redissolved in little water, and acidified with sulphuric

acid. The mixture was extracted with ether, and the extract treated for lactic acid, with zinc, etc. The zinc lactate crystallised white, weighed =0·0707 g., dried over oil of vitriol in vacuo.

The acid solution from which the lactic acid had been extracted was precipitated with phosphomolybdic acid, and the precipitate filtered off and dried =0·1270 g.

The filtrate from this was treated with barita, etc., and after treatment with ammonium carbonate and evaporation left a residue weighing 0·7500. This was redissolved in water and precipitated with basic lead acetate. Inosite lead was isolated and decomposed with hydrothion. The dry inosite, crystallised, weighed =0·1420.

The mother-liquor was freed from lead, evaporated to dryness, burnt, and the residue weighed =0·171 g.; of the metals in this, 17·67 per cent. were potassium, and 82·33 per cent. sodium.

Synopsis of the Results of Analysis of White Tissue of Human Brain.

Water expelled at 95°	70·230
Neuroplastin	8·630
Ether extracts, kephalin, lecithin, and cholesterin	11·497
Cerebrins and myelin	6·910
Insoluble in ether from buttery	0·212
Lecithin (lead)	—
Kephalin (lead)	—
Myelin (lead)	—
Water-extract, 1·403 per cent. consists of	—
Lactic acid	0·0456
Inosite	0·2151
Alkalies (carbonates)	0·1717

The separation of the ether extracts into their constituents was also carried out, and the results will be stated lower down.

C. QUANTATION OF THE ABSOLUTE AND SPECIFIC GRAVITIES OF A HUMAN BRAIN AND OF SEVERAL PARTS.

Division of the Brain.	Absolute Weight in Air.	Weight in Water.	Loss of Weight in Water.	Specific Gravity.
1. Right hemisphere	589·035	20·820	568·215	1·037
2. Left hemisphere	595·823	21·600	574·223	1·038
3. Cerebellum	135·172	5·030	130·142	1·039
4. Mesenkephalon	33·950	1·250	32·700	1·038
5. Sclerotic part	3·630	0·150	3·480	1·043
Entire Brain	1357·610	48·850	1308·760	1·037

Quantation of the Specific Gravity of White Tissue and Grey Tissue of the Human Brain.

These specific gravities were ascertained by suspension of the parts in water, etc. (grammes at 16°).

White Tissue.

Wgt. in Air.	Wgt. in Water.	Spec. Grav.
0·4870	0·0258	1·053
0·8650	0·0400	1·048
1·0746	0·0477	1·046
0·6859	0·0310	1·044
1·0479	0·0394	1·039
3·7845	0·1319	1·036
9·7000	0·2850	1·030
9·5362	0·2740	1·030

Grey Tissue.

Wgt. in Air.	Wgt. in Water.	Spec. Grav.
0·6628	0·0243	1·038
14·4592	0·3810	1·027
11·5741	0·2865	1·025

The foregoing data have been arranged in the order of decreasing specific gravities. This led at once to an inverse order in the column denoting weights in air. Only two figures out of eleven do not absolutely take the places which they would occupy if the order of increase in the first column was inverse as that in the third.

It therefore appears, what has also been confirmed by many other experiments, some to be related below, that *the specific gravity of white and grey tissue of the brain is found the higher, the smaller is the quantity of brain-tissue employed in the experiment.* Now, as the pieces of tissue which can be employed in the experiments are limited in size by the arrangement of the relative tissues in the brain, it is clear that the specific gravity quantations of brain-substance in water can only be approximately correct. The variations, no doubt, depend upon a reaction between the surface of the piece of tissue immersed and the water which surrounds it. The water takes up some soluble albumen and some salts, and the piece of brain-tissue immersed assumes a glazed appearance. It is evident from this that the greater the surface

of the piece under observation to its volume, the greater will be the effect of this source of error. The error will further be influenced by the length of time during which the piece of tissue is immersed, and consequently variations will arise, even when pieces of equal size are examined, from the interference of the accident of quicker or slower weighing. It is further doubtful whether white and grey tissues will be equally influenced by water in the same time, even when their bulks are equal. It is further not proved that either the grey or the white tissue is so homogeneous in any part of the brain as is assumed for the purposes of comparison. It is therefore clear that specific gravity estimates of brain-tissue in water have only an approximative and no absolute mathematical value. Such estimates must therefore be made with the aid of fluids of well-known specific gravity, which, while they make contact with the brain-tissue, do not provoke in it any chemical or physical changes.

Quantations of the Specific Gravities of White and Grey Tissue from different parts of the Brain.

White Tissue of Hemispheres.

(*a.*) *Quantations by the Piknometer:*

Absol. Wgt.	Subst. + Pik.	Pik. + Water.	Spec. Grav.
0·4526	61·9300	61·9120	1·041
0·2817	61·9342	61·9198	1·054

(*b.*) *Quantations by Suspension in Water:*

Absol. Wgt.	Wgt. in Water.	Spec. Grav.
0·6859	0·0310	1·044
1·0479	0·0394	1·039
3·7845	0·1319	1·036
9·5362	0·2740	1·030

Grey Tissue of Hemisphere.

(*a.*) *Quantation by the Piknometer:*

Absol. Wgt.	Subst. + Pik.	Pik. + Water.	Spec. Grav.
0·3117	61·9146	61·9039	1·039

(*b.*) *Quantations by Suspension in Water:*

Absol. Wgt.	Wgt. in Water.	Spec. Grav.
0·6628	0·0243	1·038
14·4592	0·3810	1·027

White Tissue of Cerebellum.

(*a.*) *Quantation by Piknometer:*

Absol. Wgt.	Subst. + Pik.	Pik. + Water.	Spec. Grav.
0·5900	61·9240	61·9039	1·037

(*b.*) *Quantation by Suspension in Water:*

Absol. Wgt.	Wgt. in Water.	Spec. Grav.
0·8650	0·0400	1·048

White Tissue of Mesenkephalon.

(*a.*) *Quantation by Piknometer:*

Absol. Wgt.	Subst. + Pik.	Pik. + Water.	Spec. Grav.
0·6849	61·9252	61·9039	1·032

(*b.*) *Quantation by Suspension in Water:*

Absol. Wgt.	Wgt. in Water.	Spec. Grav.
0·5128	0·0258	1·053

Synopsis and Averages of the Specific Gravities observed in the three Series of Observations detailed in the foregoing, without reference to repetition of the same numbers or to quantities on which they were observed.

I. *White Tissue.*

1. 1·054	5. 1·044	9. 1·036
2. 1·053	6. 1·051	10. 1·032
3. 1·048	7. 1·039	11. 1·030
4. 1·046	8. 1·037	

Mean specific gravity of white tissue = 1·041.

II. *Grey Tissue.*

1. 1·039	2. 1·038	3. 1·027	4. 1·025

Mean specific gravity of grey tissue = 1·032.
Specific gravity of entire brain (four parts) = 1·037.

The foregoing figures do not differ much from those accepted by other observers. The specific gravity of white tissue is the same as that commonly allowed in physiological treatises, namely 1·041, while the specific gravity of grey tissue is 1·032 instead of that commonly allowed, namely 1·034. But when it is considered that what must theoretically be assumed to be the best observations of the specific gravity of white tissue, namely those on the largest volume, do only give 1·030 as the value, while grey

tissue under the same limitation gives 1·027, it is impossible to avoid the suspicion that all specific gravity estimates hitherto made, including the foregoing, are vitiated by a fundamental fault of method, or by several faults, as above indicated. These probable faults have for the first time been observed in the course of the present researches, and there has been no time for remedying the inconveniences arising out of the observation.

In 1876, I communicated a then new method for estimating the proportion between white and grey tissue in the brain (an important physiological problem, which seems anatomically quite insoluble) by a calculation from the four factors, absolute weight of the brain, specific gravity of the entire brain, and specific gravities of each white and grey tissue. This method still has its future, if the difference between the specific gravity of white and grey tissue be not ultimately found too small.

In its execution the following formula may be used:

$$x = \frac{Pw(p-g)}{p(w-g)}, \text{ in which}$$

x = quantity of white matter.
P = absolute weight of the brain.
p = specific gravity of the entire brain.
g = specific gravity of the grey tissue.
w = specific gravity of the white tissue.

Applying this formula to the data given above, namely:

P=1358; p=1·037; g=1·032; w=1·041; then x=757·3, equal to 55 per cent. of white tissue and 45 per cent. of grey tissue in the entire brain.

About two years ago I had the happiness of conversing on this matter with the late Mr. C. W. Merrifield, a gentleman of great scientific attainments, and of clear and powerful mathematical intellect, whose recent death the State, the scientific community, his family, and his personal friends have great reason to deplore. He took an immediate interest in the question, and embodied the result of his deliberations on it in a memorandum, which I am glad to be able to record in this place. He wrote to me:

'Your problem is exactly the same as that of the estimation of gold in auriferous quartz. If for gold you read white cerebral tissue, and for silica, grey matter, the formula solves your problem.

'Let g be the specific gravity of the heavier substance (gold);
,, q ,, ,, ,, lighter ,, (quartz);
,, m ,, ,, ,, mixture;
,, x be the unknown proportionate *bulk* of gold to a unit of bulk of the mixture.

Then xg is the proportionate *weight* of gold, and $(1-x)q$ is the proportionate weight of quartz. The sum of these must give the weight of the mixture, that is—

$$xg+(1-x)q=m,$$
$$\text{or } x(g-q)=m-q,$$
$$\text{or } x=\frac{m-q}{g-q},$$

which gives the proportionate *bulk* of the gold.

'The proportionate *weight* of the gold is:

$$\frac{gx}{m}, \text{ or } \frac{g}{m}\,\frac{m-q}{g-q}.$$

Then, if $g=19$, and $q=2{\cdot}6$, while $m=9$, we have $m-q=6{\cdot}4$, $g-q=16{\cdot}4$.

$$\text{Proportionate bulk } =\frac{6{\cdot}4}{16{\cdot}4}=0{\cdot}39.$$

$$\text{Proportionate weight} =\frac{6{\cdot}4}{16{\cdot}4}\times\frac{19}{9}=0{\cdot}824.$$

'This method gives very good results when, as in the case of auriferous quartz, the specific gravities are widely different, because then a small error in the estimations of the data does not make a large error in the result. It is otherwise when the difference of density is small. For instance, suppose—

g = specific gravity white tissue $=1{\cdot}035$
q = ,, ,, grey ,, $=1{\cdot}025$
m = ,, ,, brain altogether $=1{\cdot}031$.

Then we have for proportionate *bulk* of white tissue:

$$\frac{m-q}{g-q}=\frac{0{\cdot}006}{0{\cdot}010}=0{\cdot}6\,;$$

for proportionate *weight* of white tissue:

$$\frac{g}{m}\,\frac{m-q}{g-q}=0{\cdot}6\times\frac{1{\cdot}035}{1{\cdot}031}=0{\cdot}6023.$$

'Now suppose the above data to be in error, so that in reality:

$$q=1{\cdot}034, \text{ instead of } 1{\cdot}035$$
$$g=1{\cdot}024, \quad \text{,,} \quad 1{\cdot}025$$

m being as before. Then we have for

$$\textit{Bulk} \qquad \frac{m-q}{g-q}=\frac{0{\cdot}007}{0{\cdot}010}=0{\cdot}7\,;$$

$$\textit{Weight}\ \frac{g}{m}\,\frac{m-q}{g-q}=0{\cdot}7\times\frac{1{\cdot}034}{1{\cdot}031}=0{\cdot}70204,$$

which is a very different result, an error of 1 per cent. in the two specific gravities, and both in the same direction, altering the result in the ratio of 6 : 7.'

Another method for the quantation of grey and white matter, recently proposed, is based upon the difference in the quantity of water contained in the two tissues, and expelled at 95°. We have seen that grey tissue loses 85·27 per cent., while white tissue 70·23 per cent. of water. Now, if the loss of water of the entire brain be known, and the foregoing data be physiological constants or specially ascertained in each case, the relative weights of white and grey tissue may be calculated.

D. SKETCH OF A SYSTEMATIC QUANTITATIVE ANALYSIS OF THE BRAIN.

The brain is weighed in its membranes; the latter are then carefully removed and weighed, and their weight is deducted from the weight of the brain previously found. The membranes of a human brain will be found to weigh about 60 g. The brain-tissue is then cut, or minced in a machine, and steeped in alcohol until it has become hard. It is then worked through a sieve in the manner described in the second chapter of this treatise. The brain-pulp is now exhausted with spirit of 85 per cent. strength. The boiling with spirit must be repeated until a litre of spirit boiled with the whole of the residue, filtered and distilled to dryness, leaves only an inappreciable residue. The first spirit-extracts which deposit the particular white matter are kept separate. All particles of brain-tissue on the one hand and all portions of liquid on the other must be constantly collected with scrupulous care. A human brain will leave from 100 to 120 g. of dry albuminous matter.

The alcoholic solutions of the first extractions deposit on cooling and standing *the white matter*, which amounts to 4·7 to 5 per

cent. of the mixed brain-tissues, but is mainly derived from the white tissue. (Compare on this subject the special analyses of white and grey tissue given in the previous chapter.)

Analysis of the White Matter.—It is exhausted with ether, by being shaken with it in a stoppered cylinder, and allowed to settle ; the ether is drawn off with a syphon worked by air-pressure. The white cerebrin mixture, containing the cerebrin and some phosphorised matters, remains insoluble, while all kephalin, lecithin, some myelin, all cholesterin, and some other neutral matters, an oil or ether (cerebrol) and a yellow coloured matter, dissolve. Thus we have :

Soluble in ether.	Kephalin, part combined -	All extracted.	
	Lecithin, ,, -	All ,,	
	Myelin, ,, -	Part ,,	
	Cholesterin - - -	All ,,	
	Neutral (new) matter -	Part ,,	(Phrenosterin).
	Cerebrol - - -	All ,,	
	Yellow coloured matter -	All ,,	

Treatment of the Ether Solution ; separation of its Ingredients from each other.—The ether is distilled off, the liquid residue is mixed with an alcoholic solution of acetate of lead and excess of alcohol, and boiled for some time under a reflux cooler. It is then allowed to cool and stand. The cholesterin crystallises in the upper layer of the mixture, while the kephalin lead and myelin lead remain below, insoluble. The mixture is gently warmed until cholesterin is dissolved, and again allowed to cool. This process is repeated to cause the kephalin lead and the myelin lead to become as adherent and lumpy as possible, so that when the warm spirit solution is filtered off through a filter on a hot funnel, only a minimum of the insoluble compounds may pass on to the filter. The residue is then exhausted with spirit by repeated long boiling with it. Thus we have :

Insoluble in boiling spirit.	Kephalin lead -	All precipitated.	
	Myelin lead - -	Greater part precipitated.	
Soluble in boiling spirit part.	Cholesterin - -	All.	
	Myelin lead - -	Part.	
	Lecithin - - -	All.	
	Cerebrol - - -	All.	
	Yellow coloured matter	All.	
	Neutral new matter -	All.	(Phrenosterin).

Treatment of the insoluble in boiling Spirit part (*of the Ether Extract boiled with Lead Acetate*).—This contains all kephalin lead, and some myelin lead, which have to be separated. This is done by absolute ether, in which *kephalin lead* dissolves with a red colour, while *Myelin lead* remains as an insoluble white deposit. The latter is washed with ether by decantation mainly (with the aid of a syphon and air-pressure), lastly on the filter, dried and weighed. The datum is *myelin lead part the first*. The kephalin lead solution and all ether used for washing myelin lead is distilled to dryness and the residue weighed. It constitutes kephalin lead, and contains all the kephalin which had been present in the white matter.

In each, myelin lead and kephalin lead, the metal and phosphorus are ascertained by analysis, and from the data the amounts of pure myelin and pure kephalin relatively are calculated.

Treatment of the soluble in Spirit part.—The boiling solution is mixed with as much boiling water as it will bear without becoming precipitated, and is allowed to cool slowly. *Cholesterin* (and phrenosterin ?) crystallises almost completely somewhat later, and covering the latter settles the *myelin lead*. *Lecithin* (? lead), cerebrol, yellow-coloured matter, neutral new matters remain in solution. (This mixture requires further study.)

Separation of Cholesterin from Myelin Lead.—The isolated crystallised matter is pressed, dried, and placed in absolute ether in a tall stoppered cylinder, and frequently agitated. Cholesterin dissolves, while the lead salt of myelin and a cerebroside settle as a white deposit. The extraction of cholesterin is completed by the repeated application of large volumes of ether. The united ether solutions are distilled to dryness ; the residue is dissolved in boiling dilute spirit (if not sufficiently dilute, the boiling solution must be mixed with boiling water until it becomes turbid), and the solution set to crystallise. The *cholesterin* is filtered off, dried, and weighed. The white salt which the ether dissolving the cholesterin has left insoluble, is *myelin lead*, and a cerebroside. This is dried and weighed. It gives the purple reaction with oil of vitriol alone on standing, immediately with sugar-syrup.

The *spirit mother-liquor of the cholesterin*, after concentration, may yet yield a minute quantity of cholesterin, but does not contain much else, colouring-matter excepted. It may be added to

the principal mother-liquor, from which cholesterin and myelin lead were originally deposited.

Solution in Spirit of Lecithin, Cerebrol, Yellow Colouring Matter, Neutral Matter, and some Cholesterin.—Lecithin does not remain combined with lead in watery spirit. It cannot be separated from the rest of cholesterin except by precipitant reagents, or by chemolysis, by which it is decomposed. Its quantation has been found to be effected with the greatest approximation to truth by the following process: The spirit solution containing the matter just named is heated until all the spirit is evaporated. The residue is then treated with boiling water, in which it hardens and becomes granular. (In cold water it swells and becomes pasty, so that it cannot be separated from its mother-liquor.) The hot water is now decanted, and replaced by new; the mixture is allowed to cool, heated again to make the solids curdle, and the water is again decanted. This is repeated until the decanted water is free from lead.

Chemolysis of the Lecithin, etc., Mixture with Barita.—The mixture as described is now mixed with the necessary quantity of barita hydrate and water, and chemolysed in a closed platinum tube under pressure at 125° for at least six hours. The contents of the tube are extracted firstly with hot water, which removes excess barita, *glycerophosphate of barium* and *neurin*. The solution is neutralised with carbonic acid, and the filtrate is evaporated. When suitably concentrated, the addition of absolute alcohol to it precipitates all *barium glycerophosphate*, while the filtrate contains all *neurin*. The solution is neutralised by hydrochloric acid in excess, and the addition to it of alcoholic platinic chloride precipitates all neurin as platino-chloride hydrochlorate. Both salts, the barium glycerophosphate and neurin double salt, are dried and weighed.

Products of the Barita Chemolysis of the Lecithin Mixture which are Insoluble in Water.—These are extracted with boiling spirit. The concentrated spirit solution deposits yet some cholesterin and a small quantity of a barium salt. These are removed by the filter. The cholesterin is separated from the barium salt by ether. The spirit solution now retains the bodies above mentioned—namely, two neutral crystallised bodies (which are here noticed for the first time), and perhaps some cerebrol and yellow colouring-matter. The two new bodies crystallise out of

the absolute alcohol solution. Ether separates the second crystallised body from the first; the latter is recrystallised from spirit. A thick mother-liquor remains, which requires further qualitative examination. It is dried and weighed, and placed in the account as *last product of chemolysis of lecithin mixture.*

That part of the product of the chemolysis of the lecithin mixture which is insoluble in water as well as boiling spirit contains *the barium salts of the fatty acids* produced from the chemolysed *lecithin*, as well as from the chemolysed ethylic ethers of fatty acids resulting from a previous partial decomposition of lecithin under the influence of heat and alcohol only. As they contain barium carbonate, they have to be reconstituted in a pure state, and the different fatty acids have then to be separated. This is best done by decomposing the salts in water with hydrochloric acid, and extracting the fatty acids with ether. The ether is distilled off, the fatty acids are dissolved in watery ammonia, and precipitated as lead salts by lead acetate. The lead salts are dried, powdered, and exhausted with ether. The ether solution on distillation leaves *lead oleate*, while the salt insoluble in ether will be found to be mainly *lead palmitate* or *margarate*, with only little, if any, *stearate.* From the oleate and palmitate, with the aid of the neurin and glycerophosphate, the amount of lecithin originally present is easily calculated.

Treatment of the Alcoholic Solution which has deposited the White Matter.—This solution, and all alcoholic extracts of the albuminous part obtained until it is exhausted, are distilled together to a convenient state of concentration, and allowed to cool. A matter is then deposited which, from its consistency, has been called '*buttery*,' and which consists of cholesterin, lecithin, kephalin, myelin, and some other ingredients. This is separated from the liquid by filtration, and analysed as will be described.

Treatment of the Concentrated Alcoholic Solution which has deposited the Buttery Matter.—This solution is evaporated on the water-bath until all alcohol has disappeared. The last portions of phosphorised matters and cholesterin, together with small quantities of oily ethers, then float on the watery liquid and adhere to the evaporating-dish. This product is termed the '*last oily.*' It is most advisable to separate this from the watery solution without the employment of a filter, and after slightly rinsing with distilled water, to add it to the buttery matter for further analysis.

Analysis of the United Buttery and Last Oily Matters.—To these matters, after they have been dissolved in a sufficiency of hot alcohol, the lead process, as described in the paragraph relating to the ether extract of the white matter, may at once be applied. Kephalin lead and myelin lead are precipitated; lecithin, cholesterin, and some myelin lead remain in solution, together with other matters to be described. Thus we have—

Precipitated from and insoluble in boiling spirit - - -	Kephalin lead (all). Myelin lead (part).	
Soluble in boiling spirit, part deposited on cooling - -	Cholesterin Myelin lead and a cerebroside	deposited on cooling.
	Lecithin. Cerebrol. Yellow matter. Neutral matters.	

The kephalin lead and myelin lead are separated from each other by ether as above described, and their quantities weighed. The lead contained in the respective preparations is ascertained by a special quantation of the phosphorus and the lead, and from these data the actual amount of each of the free phosphorised principles is calculated.

The myelin lead and cholesterin deposited from the hot alcohol are also separated by ether, and the products weighed. The cholesterin may be weighed as residue from the ether solution after distillation of the ether from a tared flask, or it may be recrystallised from very dilute spirit, and weighed in the crystallised state. When thus recrystallised, it is so pure that its melting-point is mostly at 145°.

The cold spirit solution, containing lecithin, cerebrol, yellow matter, neutral new matter, and mostly a residue of cholesterin, together with some lead acetate, has now to be chemolysed, so that its ingredients can be ascertained from the products of decomposition. The spirit is first evaporated, and the residue heated with water, which dissolves the acetate of lead and any impurity soluble in water. This purification with hot water (cold water has to be avoided, as it makes the residue swell and present a semi-mucilaginous state) is repeated until the water is free from lead. The residue is now treated as follows:

Chemolysis of the Last Residue of the Buttery and Last Oily Matter

which was not precipitated by Lead Acetate.—The necessity for this process arises from the fact that the bulk of the lecithin cannot be separated from the last traces of cholesterin and from the small quantities of ether formed during the long processes with alcohol. There are, moreover, matters present, such as the neutral new matters, which are all soluble in ether as well as alcohol, and do not combine with reagents in such a manner as to become insoluble while the other bodies remain soluble, or inversely.

The mixture as described is chemolysed in the same manner as the residual mixture from the lead-precipitates from the ether-extract of white matter described above. It is mixed with the necessary quantity of barita hydrate and water, and heated in a closed platinum-tube under pressure at 125° for at least six hours. It may also be boiled in an open platinum dish, with frequent renewal of the water, for at least twelve hours; but the process is liable not to be complete, as the fatty acid salts formed may enclose portions of lecithin and protect them from the influence of the barita. Smaller quantities of matter may be enclosed in glass tubes and sealed, and then heated to 125°, surrounded by water in a closed brass tube. This last precaution is necessitated by the fragility of the glass, if unprotected. The employment of the platinum tube is preferable, owing to its simplicity. The contents of the tube are extracted with hot water, which removes excess of barita, *glycerophosphate of barium and neurin.* These are isolated as above described—the glycerophosphate by alcohol, and the neurin by platinic chloride.

The remaining solid products of the chemolysis, which are insoluble in water, are extracted with boiling spirit. In this all cholesterin, and other matters not yet fully identified, and a little barium salt of a fatty acid dissolve, while the barium salt of the fatty acids produced by the chemolysis from lecithin and cerebrol remain insoluble.

The spirit solution, when sufficiently watery, deposits all cholesterin, which is dried and weighed; it retains in solution the neutral matters alluded to. This last mother-liquor is evaporated to dryness and weighed, and the product is entered into the record of the analysis as crystallisable undetermined products.

The barium salts of the fatty acids, oleate and margarate or palmitate, are decomposed with hydrochloric acid and water under

ether, and the liberated fatty acids are then combined, first with ammonia, next with lead. From the dry mixture of lead oleate and margarate the former is extracted by ether. The *margarate* remains as a white substance, which is easily weighed; the *oleate* is best weighed as residue of the ether solution distilled from a tared flask.

In calculating the amount of lecithin from the oleic and margaric acids obtained as lead-salts, and comparing these data with those obtained by calculation from the quantities of glycerophosphoric acid and neurin, the following circumstances have to be borne in mind:

Of the lecithin present in the brain, and extracted by the alcohol, a part is already decomposed during the chemical operation. We shall see below how an excellent *quantation of the lecithin* can be made upon any portion of brain, provided it is not intended to estimate many other or all the ingredients of the extract obtained therefrom. But in the course of a complete analysis of a single brain, such as is here described, the complete quantation of the lecithin as such is not feasible, on account of the decomposition just alluded to. This decomposition causes a loss of neurin and glycerophosphoric acid on the one hand, which remains in the watery mother-liquor containing the principles of the brain soluble in water, and a loss of fatty acids on the other, which combine with alcohol and form ethers. These ethers are, of course, again decomposed during the barita chemolysis, so that ultimately the whole of the fatty acids which were present in the form of lecithin are obtained as barium salts. It follows, therefore, that when the quantities of fatty acids found are compared with the quantities of neurin and glycerophosphoric acid, equivalent for equivalent, there will be *an excess of fatty acid* over the neurin and glycerophosphoric acid. It is therefore necessary to take the fatty acids as the basis of calculation for the amounts of lecithin present in the original extract, and the neurin and glycerophosphoric acid only as subsidiary aids for the determination of the minimum and the control against accident.

Separation of the Ingredients of the Buttery Matter by a Process in which Caustic Barita is employed.—In this process barita takes the place of lead which is employed in the process just described. The results are in the main analogous. The mother-liquors will have to be chemolysed with more barita, as in the previous case,

but the process will not be delayed by the necessity of removing the excess of lead acetate.

The buttery matter is dissolved in a sufficient quantity of hot spirit, and filtered hot. To the hot solution, hot barita water (saturated in the cold) is now added, while the mixture is kept boiling. A precipitate separates and becomes adhesive. *The solution* is decanted and filtered boiling. *The precipitate* which remains insoluble is exhausted with boiling spirit, dried and treated with ether. *Kephalin-barium* goes into solution, while a white salt (*myelin-barium* and small quantities of *barium salts of fatty acids*) remains insoluble. The hot spirit solution, on cooling, deposits a white precipitate, and then is almost free from ingredients. The precipitate is isolated, dried, and exhausted with ether. This solution of ether contains all cholesterin, and a mere vestige of kephalin. The white precipitate contains, firstly, a body soluble in boiling spirit, and deposited from it in needles (curved needle body), and a body which is now, after removal of the bodies soluble in ether, insoluble in boiling spirit, and contains much barium and phosphorus.

The kephalin-barium as precipitated from human buttery by barita-water in the above process is not yet a pure compound, as was shown by the following tests of a specimen. It was insoluble in boiling spirit, easily soluble in ether, twice precipitated by absolute alcohol, and dried over oil of vitriol at 70°. It contained 23·30 per cent. Ba. and 4·67 per cent. P.

The amount of barium found corresponds approximately to a dibarium kephalin, which requires 24·77 per cent. Ba. But the amount of phosphorus is in excess of that theory, which requires 2·9 per cent. P, and must be left unexplained. Dibarium-kephalin has an analogue in a diplumbic kephalin, which I have described in the chapter relating to kephalin.

Analysis of the Cerebrin Mixture.—This mixture consists of a number of well-defined immediate principles, which belong mainly to three distinct categories.

(1.) *Cerebrosides* or bodies of the glucoside type, which contain as constitutional base a peculiar sugar, *cerebrose.* The type of these bodies, *phrenosin,* forms the main quantity of the ingredients of the mixture. It is insoluble in cold absolute alcohol. *Kerasin* is soluble in much cold absolute alcohol, at least for some time, and is deposited slowly on standing in a semi-crystalline form.

A body crystallising apparently in curved needles, bregenin, is permanently soluble in alcohol. These bodies do not combine with lead when it is added as acetate to their solution in spirit. But there are a number of cerebrosides which do combine with lead when it is added as acetate to their spirit solution: of this class is *cerebrinic acid*, and *spherocerebrin*, and the three bodies accompanying spherocerebrin as described in the article on the cerebrinacides.

(2.) *Phosphorised bodies*, which, owing to some of the fatty acids contained in them being identical with or nearly allied to the fatty acids contained in the cerebrosides, have the same or very nearly the same solubility in alcohol and other solvents as the cerebrosides, and therefore follow them pertinaciously: *sphingomyelin* and *apomyelin*.

(3.) *Sulphurised bodies*, of which a preliminary description has been given in a previous chapter.

The cerebrin-mixture also always contains varying quantities of bases, particularly potash and soda.

The cerebrosides being mostly neutral bodies, having no affinity for either acid or alkali, can be isolated with solvents only. Of the cerebrin-mixture an elementary analysis should be made, and its results stated in atoms with sulphur as unit, and again with phosphorus as unit. This will at once show the proportions of atoms to each other, and be the chief control of the processes of quantitative separation to be undertaken afterwards.

The cerebrin mixture, dissolved in alcohol, may then be treated with lead acetate and a little ammonia, and the precipitate may be exhausted with boiling spirit. This process separates the mixture of cerebrosides into the two categories described above under (1). The sulphurised bodies remain principally in the lead precipitate, the phosphorised principles distribute themselves over precipitate and solution. The separation and quantation of these matters requires further study.

The nitrogen in the cerebrosides is probably all present in a form, which by chemolysis with barita or sulphuric acid, sufficiently long continued, will appear as sphingosin. Thus neurin on the one, and sphingosin on the other hand, will probably be the only nitrogenised nuclei to be isolated by chemolysis. They are certainly the principal ones as regards quantity; should the sulphurised bodies contain any nitrogen and that in a particular

form, then the quantity of this particular product would be much below the quantities of the products just mentioned.

E. A PRELIMINARY EXPERIMENT FOR THE QUANTATION OF CONSTITUENTS OF AN ENTIRE HUMAN BRAIN.

Of the *left hemisphere*, which when entire weighed 596 g., 460 g. were taken for the following quantations. The tissue was comminuted and exhausted with boiling spirit, etc., and yielded the following educts:

Albuminous matters =35·06 g., equal to 7·62 per cent. of tissue.

White matter, deposited from spirit, 21·93; the same after extraction with cold ether (cerebrin-mixture) dry= 12·28 g. Soluble in ether, 9·65 g. These *cerebrosides*, etc., boiled with absolute alcohol, gave—

(*a.*) Less soluble cerebrins, phrenosin, etc., deposited immediately 8·11 g.

(*b.*) More soluble ones, kerasin, etc., deposited after days 0·56 g. And left insoluble stearoconote, albumen, and paper fibres 0·78 g.

Of this last item 0·53 were soluble, 0·25 insoluble in hot benzol. The insoluble in benzol part contained a body which left a black ash on combustion, and contained much phosphorus. It was an earthy compound of a phosphorised body. It gave a brownish red, but no genuine oleo-cholide *reaction.*

The alcoholic solution from the cerebrins =340 cc. was divided in two equal halves of 170 cc. each. To one half platinic chloride was added as long as a precipitate was produced. The precipitate, containing a phosphorised body, weighed 0·85 g., and contained 9·58 per cent. Pt; it yielded further 3·13 per cent. P. To the second half of the alcohol solution cadmic chloride was added, and the precipitate obtained weighed 0·8124 g.; it yielded 13·27 per cent. Cd and 3·42 per cent. P.

Of the matters dissolved in spirit, and not precipitated by these alkaloid reagents, 0·3619 g. were yet precipitated by water.

The ether-extract from the cerebrin mixture weighed 9·65 g.

The buttery matter, soluble entirely in cold ether =21·65 g.

The last oily matter, treated with lead acetate, yielded lead precipitates which together weighed 11·8450 g.

Out of these there was obtained *lecithin* =2·8995 g. (This was

easily soluble in cold absolute alcohol, and gave the characteristic tests with platinic and cadmic chloride.)

Further *kephalin* lead = 0·8435 g. = 0·5659 g. *kephalin; and myelin* lead = 6·6080 g. = 5·2041 *myelin.*

The last watery extract gave a phosphomolybdate precipitate of alkaloids, which weighed dry 3·7084 g. It yielded further 1·56 g. *zinc lactate* dried in vacuo over oil of vitriol = 1·0074 g. *pure sarcolactic acid.*

The inosite amounted to 2·5335 g.

The undefined organic extractives weighed 2·7822 g.

The salts, as carbonates, weighed 1·7218 g. Of these 0·39 were potassium = 0·745 KCl, and 0·39 sodium = 0·9844 NaCl. The salts as chlorides weighed 1·7294 g., and out of this mixture 2·4610 g. $PtCl_4(KCl)_2$ were obtained.

In the calculation of kephalin, myelin, and lactic acid, the following formulæ were used, which should be considered as provisional, and will have to be tested by further, or supplemented by direct analysis of the products :

Kephalin, molec. weight = 836 ; kephalin lead = $C_{42}H_{75}NPO_{13}Pb_2$ M.W. = 1246.

Myelin, M.W. = 760; myelin lead = $C_{40}H_{73}PbNPO_{10}$, M.W. = 965.

Sarcolactic acid $C_3H_6O_3$, M.W. = 90.

Zinc sarcolactate dihydrate, $C_6H_{10}ZnO_6 + 2H_2O$, M.W. = 279.

The buttery matter which had been soluble in ether (21·6450 g.) was boiled with barita and yielded 6·7 g. of cholesterin, with 16·5 g. of barium salts of fatty acids. The latter contained oleate (as shown by the oleo-cholide reaction), much phosphorus, and a small quantity of a body which was soluble in ether. But the complete extraction with ether was impracticable, as the turbid liquid would neither settle nor allow itself to be filtered. This difficulty is not rarely met with when dry barium or lead salts of brain educts are subjected to ether treatment for the extraction of some ingredient.

The ether extract from the cerebrin mixture, containing the kephalin and cholesterin, was treated with alcoholic lead acetate and filtered boiling. The insoluble residue of kephalin lead weighed 2·6410 g., which, assumed to contain 2Pb, is equal to 1·772 g. free kephalin.

The solution in spirit of the cholesterin, etc., was evaporated, and the residue freed from lead acetate by hot water. The cholesterin

was then re-crystallised and weighed. When the cholesterin thus obtained was re-dissolved in ether, a white matter remained insoluble, weighing 0·2680 g., and being entirely combustible on platinum foil.

Quantation of the Ingredients of the Right Hemisphere.

The total hemisphere, with the membranes, weighed 589 g.; after removal of membranes, 564 g.; of this quantity, 465 g. were employed in the following quantations:

The albuminous substance amounted to 35·68 g., equal to 7·66 per cent. of the hemisphere tissue.

The white matter deposited from spirit dry was = 18·60 g.

The ether-extract from this = 6·97 g.

The cerebrins insoluble in ether = 11·63 g.

The buttery matter (= 21·66 g.) in alcohol was treated with lead acetate, and boiled. The precipitates insoluble in boiling alcohol were suspended in ether for separation, but the cylinder containing the mixture breaking spontaneously, the quantation was lost. In the table this void is filled up by data obtained from the data concerning the left hemisphere by calculation.

The inosite was obtained in two portions, one with neutral acetate (it was not previously known that inosite was so precipitated), another with basic, together about 0·43 g. The lead having been removed from the liquid with sulphuric acid, the *lactic acid* was extracted and formed into zinc salt. It weighed, dry at 100°, 0·87 g. = 0·64 sarkolactic acid.

Alkaloids were precipitated from the mother-liquor by phosphomolybdic acid. The precipitate weighed 3·8 g., and contained 1·30 g. mixed alkaloids.

The mother-liquor was treated with barita, etc., evaporated, and the residue burnt. The *indefinite extractives* amounted to 2·63 g.

Of potassium 0·25 g., of *sodium* 0·40 g., were obtained.

The hot mother-liquor of the buttery matter treated with lead acetate (of which operation the insoluble precipitates were lost as above described), on cooling deposited myelin lead and *cholesterin*, weighing, when dry, 6·99 g. The myelin weighed 5·22 g.

The spirit filtrate, containing *lecithin and little cholesterin*, after evaporation, left a residue weighing 8·13 g.

The remaining bodies containing cholesterin were chemolysed

with barita, and gave *cholesterin*=8·93 g. pure, and 8·80 g. *barium salts of fatty acids.*

Quantation of the Ingredients of the Cerebellum.

The total weight of the fresh cerebellum with membranes was 135 g., and after removal of membranes, etc., 124 g. were analysed.

The albuminous matter amounted to 11·3809 g.=9·17 per cent. of cerebellum.

The white matter from first spirit was=1·81 g. Of this, 0·2185 g. were soluble, 1·6645 g. insoluble in ether =cerebrins.

The filtrate from the white matter was evaporated and treated directly with lead acetate. There were obtained kephalin lead =1·97 g.; myelin lead 1·65 g.; in the solution, cholesterin with lecithin and myelin lead =3·26 g.; a second portion of myelin lead with lead salt insoluble in ether containing *inosite* =0·05 g. This inosite having been precipitated by neutral lead acetate (a reaction hitherto unknown) made the quantation of this body inaccurate. It amounted probably to 0·66 g. for the entire cerebellum.

The lactate of zinc, dry at 100°, weighed 0·19, equal to 0·1352 g. sarkolactic acid.

The alkaloids, precipitated by phosphomolybdic acid, weighed in combination 1·62 g., free 0·6920 g.

The extractives, mixed with the salts, weighed 1·55 g.

The alkali salts consisted of 0·01 *potassium* and 0·02 *sodium.*

The mixture of cholesterin, lecithin and myelin lead, above described as weighing 3·26 g., was boiled with barita and a little spirit for three hours. The watery solution of neurin glycerophosphate and excess of barium was separated from the insoluble matter. The cholesterin was extracted by boiling spirit, and weighed 1·95 g. Its melting-point was 145°. The *barium salts of the fatty acids* weighed 2·56 g.

Quantation of the Constituents of the Mesenkephalon.

The mesenkephalon and medulla oblongata weighed 34 g., without the membranes 33 g.

The albumen amounted to 2·48 g.=7·5 per cent. of the mesenkephalon.

The white matter weighed 0·64 g.; of this 0·03 were soluble in ether, 0·56 insoluble.

The buttery matter yielded 0·67 kephalin lead.

The lactate of zinc weighed 0·11, air dry, equal to 0·07 *lactic acid.*

The rest of the matters soluble in water were not estimated on account of their small quantity.

The data thus far ascertained have been arranged in the following table. They claim to be minima only, and with improved processes somewhat larger quantities will probably be found. Blanks are left where the quantations could either not be made, or were unsatisfactory when the products were tested.

The experience gained by this analysis has shown that the division of all the educts of a brain into five primary categories is practical. They are (1) albumen, (2) white matter, (3) buttery matter, (4) last oily matter, (5) matters soluble in water. Of these, the last oily and the buttery matters may be treated together for the separation of their ingredients, when the cerebellum or mesenkephalon are concerned. When derived from the hemispheres these products are more conveniently kept apart.

The table contains about 130 data. But it will be seen that the cerebrosides, *e.g.*, occupy only one column (col. 6), whereas probably ten columns will be required to register the quantities of various specific bodies of which the insoluble in ether part of the white matter is composed. I estimate, therefore, that the quantitative analysis of one brain will involve the production and weighing of about 300 definite bodies or compounds. Each of the four divisions of the brain, and each of the two varieties of tissue, the white and the grey, would thus require about fifty quantations for chemical characterisation.

The loss registered in the table appears at first sight enormous. One part was incurred by diffusion from the parts immersed in water for the estimation of the specific gravities as described in a previous chapter. This quantity was 1·65 g., as ascertained by evaporation of the water and weighing of the dry residue. An uncertain part of weight was lost by evaporation. But the greatest part was lost in the course of the anatomical separation of white and grey matter, in the course of comminution, and transfer from filter to filter and vessel to vessel. This loss may be much diminished by improved apparatus, but in the present case it imports only a slight degree of inaccuracy into the general result of the analysis, as its effect has been supplemented by a proportional calculation.

TABLE SHOWING RESULTS OF THE QUANTITATIVE ANALYSIS OF A HUMAN BRAIN.

Weights in grammes; third decimals below 5 omitted, above 5 added as 1 to second decimals. Membranes weighed 58 g.

—	1. Weights with Membranes.	2. Weights without Membranes.	3. Quantities analysed.	4. Albumen.	5. White Matter.	6. W. M. insoluble in Ether.	7. W. M. soluble in Ether.	8. Buttery Matter.	9. Kephalin Total.	10. Myelin Total.	11. Lecithin Total.	12. Cholesterin Total.	13. Inosite.	14. Lactic Acid.	15. Hypoxanthin and Alkaloids.	16. Indefinite Extractives.	17. Potassium.	18. Sodium.
Right hemisphere	589	564	465	35·68	18·60	11·63	6·97	21·66	3·34	5·22	5·90	8·93	0·43	0·64	1·30	2·63	0·25	0·40
Left hemisphere	596	570	463	35·06	21·93	12·28	9·65	21·65	—	5·20	—	6·99	0·38	1·00	1·06	2·78	0·39	0·39
Cerebellum	135	129	124	10·94	1·88	1·66	0·22	3·26	1·29	3·76	2·97	1·95	0·05	0·13	0·67	1·49	0·01	0·02
Mesenkephalon and medulla oblongata	34	33	33	2·48	0·64	0·56	0·03	—	0·45	0·23	0·41	1·01	0·06	0·07	—	0·73	0·01	0·03
White tissue	—	—	66	5·70	6·98	4·56	2·42	5·31	0·06	0·16	0·48	2·15	0·14	0·05	—	0·44	0·03	0·04
White tissue for quantation of water	—	—	9·2	0·80	0·97	0·64	0·33	0·74	0·002	0·02	0·07	0·30	0·02	0·006	—	0·06	0·004	0·006
Grey tissue	—	—	46	3·50	0·30	—	0·30	—	0·15	0·02	0·73	0·90	0·69	0·04	0·10	0·31	0·01	0·09
Grey tissue for quantation of water	—	—	6·2	0·46	0·04	—	0·04	—	0 02	0·003	0·09	0·12	0·09	0·006	0·01	0·04	0·001	0·01
Loss in operations	—	—	86·6	6·58	4·12	2·24	1·88	4·14	0·63	0·69	1·10	1·86	0·08	0·20	0·21	0·52	0·07	0·07
Totals	1354	1296	1296	101·20	55·46	33·57	21·84	56·76	5·94	15·30	11·75	24·21	1·94	2·14	3·35	9·00	0·78	1·06

I have no doubt that by continued study the quantitative analysis of the brain may attain a very high degree of accuracy. This belief is based upon a number of compounds and processes described in the chapters relating to the different principles, which may here be summarily referred to, although there has not yet been time for giving them places in a systematic analytical process.

The *kephalins* may be combined with lead, barita, or cadmium chloride. All these compounds are soluble in ether, insoluble in alcohol.

The *lecithins* may be combined with cadmium chloride. These compounds are insoluble in ether, insoluble in cold alcohol, soluble in boiling alcohol, soluble in cold benzol.

The *myelins* must be combined with lead; in that state they are insoluble in alcohol and ether, hot or cold.

The *paramyelins* may be combined with cadmium chloride; in that state they are soluble in hot benzol, insoluble in cold, insoluble in ether.

The *amidomyelins* may be combined with cadmium chloride; in that state they are insoluble in hot or cold benzol, and insoluble in ether.

The *sphingomyelins* are, as cadmium chloride salts, soluble in hot benzol, and soluble in much cold benzol; benzol, therefore, offers no facilities for their separation.

The *assurins* are not precipitated from alcohol by either lead acetate or cadmium chloride, but by platinic chloride.

Phrenosin and *kerasin* are soluble in boiling spirit, insoluble in cold; the later deposition of kerasin affords means for its separation. They are insoluble in ether, and do not combine with lead, or cadmium chloride.

Krinosin is soluble in hot ether, insoluble in cold; soluble in boiling alcohol.

Bregenin is soluble in cold ether and cold alcohol. Neither krinosin nor bregenin combines with lead, or with cadmium chloride.

The *cerebrinacides* combine with lead, and as lead compounds are insoluble in boiling alcohol; a part of the lead compounds is soluble, another insoluble in benzol.

Many of the phosphatides can also be combined, like assurin,

with platinic chloride. If we add to these reagents the means furnished by limited chemolysis, and by complete chemolysis, it will be seen that the quantation of any one of the well defined ingredients is now feasible. But I have no doubt that specific solvents, as well as precipitants, will be found for all the brain educts or their compounds. Thus a few trials with acetone have shown that it will be useful in the separation of the cerebrosides ; in a similar manner chloroform will be an occasionally useful solvent.

The power of mercuramin for the removal of all acids from any solution, and their recovery from the precipitate, gives to the analyst a power which was undreamed of a few years ago. The power of phosphomolybdic and phosphotungstic acids for the isolation of alkaloids has made their extraction amenable to pure reagents. And we can see from the behaviour of many of the educts with even commonplace reagents that amongst them there are at least some which will furnish means for stoichiometric proceedings.

When the normal composition of the brain shall be known to the uttermost item, then pathology can begin its search for abnormal compounds or derangements of quantities. Thus the amyloid degeneration is specific to brain and nerve-tissue, and can be considered hypothetically as a reduction of an ingredient of decomposed cerebrosides. This hypothesis has the advantage that it is as yet the only one which can be made regarding this remarkable disease. I believe that the great diseases of the brain and spine, such as general paralysis, acute and chronic mania, melancholy, and others, will all be shown to be connected with specific chemical changes in neuroplasm, the products of which cannot be more complicated than the chemolytic products of the educts; they need, however, not be identical with chemolytic products, but may be new morbid products. Here is a field for inquiry of the possession of which the guardians of refuges for the insane will hereafter, I have no doubt, endeavour to make good use.

The knowledge of the composition and properties of neuroplasm and of its constituents will also aid us in devising modes of radical treatment in cases in which at present only tentative symptomatic measures are taken. In short, it is probable that by

the aid of chemistry many derangements of the brain and mind, which are at present obscure, will become accurately definable and amenable to precise treatment, and what is now an object of anxious empiricism will become one for the proud exercise of exact science.

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